EXPRESSION OF E-CADHERIN, CYTOKERATIN-14 AND CYTOKERATIN-19 IN TOOTH GERM AND AMELOBLASTOMA

A Dissertation submitted in
partial fulfillment of the requirements
for the degree of

MASTER OF DENTAL SURGERY

BRANCH – VI

ORAL PATHOLOGY AND MICROBIOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 032

2012 - 2015

CERTIFICATE

This is to certify that **Dr. R. MUDASSAR SHARIEF**, Post Graduate student (2012–2015) in the Department of Oral Pathology and Microbiology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 has done this dissertation titled **"EXPRESSION OF E-CADHERIN, CYTOKERATIN-14 AND CYTOKERATIN-19 IN TOOTH GERM AND AMELOBLASTOMA"** under my direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch – VI) **Oral Oral Pathology and Microbiology** degree examination.

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DECLARATION

I Dr. R.Mudassar Sharief do hereby declare that the dissertation titled **"Expression of E-Cadherin, Cytokeratin-14 and Cytokeratin-19 in tooth germ and ameloblastoma**" was done in the Department of Oral Pathology and Microbiology, Tamil Nadu Government Dental College and Hospital, Chennai-600003. I have utilized the facilities provided in the Government Dental College and Hospital and Institute of Obstetrics and Gynaecology, Madras Medical College, Egmore, Chennai 600 008 for the study in partial fulfillment of the requirements for the degree of Master of Dental Surgery in the specialty of Oral Pathology and Microbiology (Branch VI) during the course period 2012-2015 under the conceptualization, design and guidance of the Principal investigator, **Prof. and Head Dr. I. PONNIAH, MDS.**

I declare that no part of the dissertation will be utilized for gaining financial assistance, for research or other promotions without obtaining prior permission from the Tamil Nadu Government Dental College and Hospital, Chennai-3.

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and

Dr. I. PONNIAH MDS., aged 48 years working as Professor and Head of the Department of Oral Pathology and Microbiology at the college, having residence address at Plot No. 164E, 7th Cross Street, "Ring Road Housing Sector", Madhavaram in Chennai 600 060 (herein after referred to as the 'Researcher and Principal investigator')

and

Dr. R. MUDASSAR SHARIEF aged 31 years currently studying as Post Graduate student in the Department of Oral Pathology and Microbiology (herein after referred to as the 'PG/Research student and Co- investigator').

Whereas the 'PG/Research student as part of his curriculum undertakes to research on the study titled **"Expression of E-Cadherin, Cytokeratin-14 and Cytokeration-19 in tooth germ and ameloblastoma**" for which purpose the Researcher and Principal investigator shall act as Principal investigator and the College shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator

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1.

2.

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Ref.No.0430/ DE/ 2010 Date: 21.02.2014

Title of the work: "Expression of Calretinin, Cytokeratin-14 and Cytokeratin-19 in Tooth Germ and ameloblastoma"

Principal investigator: **Dr.R.Mudassar Sharief**
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The request for an approval from the Institutional Ethical Committee (IEC) considered on the IEC meeting held on **29.01.2014** at the Principal's Chambers Tamil Nadu Government Dental College and Hospital, Chennai – 3

"Advised to proceed with the study"

The Members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above , submitted by the principal investigator.

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CHAIRMAN

25th March 2013
Chennai.

From

The Professor and Head,
Department of Oral Pathology
Tamil Nadu Government Dental College and Hospital
Chennai - 600 003

26/4/13

To

The Director,
Institute of Obstetrics and Gynaecology
IOG Campus
Chennai - 600 008

Madam,

Subject : Seeking permission to collect foetus specimens regarding.

For the purpose of making study slides on different stages of tooth development for Undergraduate and Postgraduate students of Department of Oral Pathology, Tamil Nadu Government Dental College and Hospital, we are in need of foetus specimens. I kindly request you to grant us permission to collect the same from your institution.

26/04/13

Yours sincerely,

March 24, 2013.

Dr. I. PONNIAH, MDS.,
(Reg No : 1667)
Professor & Head
Department of Oral Pathology
Tamilnadu Government
Dental College

ACKNOWLEDGEMENT

My sincere thanks to **Prof. Dr. S. Premkumar, MDS**, Principal [FAC], Tamil Nadu Government Dental College and Hospital, Chennai 600 003, for constant support and encouragement.

I also sincerely thank **Prof. Dr. K. S. Gamal Abdul Nasser, MDS, PhD.,** former Principal, Tamil Nadu Government Dental College & Hospital, Chennai 600 003, for not only granting me permission as Chairman of the Institutional Ethical Committee (IEC) to undertake this study, but also for providing Olympus BX43 Research Microscope without which the photomicrographs illustrated in this study would not have reached its final form.

I would like to thank all the members of the Institutional Ethical Committee (IEC), Tamil Nadu Government Dental College and Hospital, Chennai 600 003 for their critical scientific comments and granting permission to undertake this study.

I extend my heartfelt gratitude to the **Director/Professor** and other faculties of the Institute of Obstetrics and Gynaecology, Madras Medical College, Egmore, Chennai 600 008, for graciously according permission to utilize unclaimed human foetus for this study.

I owe thanks to my co-PG, Dr. S. Kuzhali for encouragement and support. I also thank other postgraduate students of my department (Dr. Parthiban, Dr. Manjula Marandi, Dr. Azeema Zulaika and Dr. Madhu Narayan) for their help and support. I also thank my former senior postgraduate student Dr. Jaisanthosh Manikandan, MDS for his timely help at the beginning of this study.

I thank **Dr. Mukul Vij, MD,** General Pathologist, Global Hospital, Chennai 600 100, for giving me permission to undergo training in immunohistochemistry. I also thank Mr. Kavivanan, the chief laboratory technician, Global Hospital, Chennai 600 100, for his laboratory assistance during this study.

My sincere thanks to Mrs. Meenakshi and Mrs. Hilda Margaret the laboratory technicians at the Tamil Government Dental College and Hospital, Chennai 600 003, for their laboratory assistance during this study.

I also express my sincere gratitude for the kind encouragement showered on me during my postgraduate course by Dr. R. Bharathi, MDS, Professor of Oral Pathology, Dr. S. Gnanadeepam, MDS, Associate Professor of Oral Pathology, and Dr. M. P. Sumathy, MDS, Associate Professor of Oral Pathology, TN Government Dental College & Hospital.

I also thank Dr. Dhanalakshmi, Tutor/Assistant Professor of Oral Pathology, MDS, Dr. Shanthi, MDS, Tutor/Assistant Professor of Oral Pathology, and Dr. J. Jayalakshmi, MDS, Tutor/Assistant Professor of Oral Pathology, for their support and encouragement.

I thank **Dr. I. Ponniah, MDS**, Professor and Head of Oral Pathology, Tamil Nadu Government Dental College and Hospital, Chennai for his help in the conceptualization, design, and as well as for his guidance as Principal Investigator during all stages of this study.

I would fail in my duty if I fail to recognize all the qualified teaching faculty who had served in the department in the order as found below; Prof. Dr. R. Viswanathan, Prof. T. R. Saraswathi, Dr. Shantha Bharathan, Prof. V. L. Indirani, Prof. R. Chandrabai, Prof. Shaheen Ahmed, Dr. I. Ponniah, Dr. M. R. C. Rajeswari, Dr. R. Bharathi, Dr. S. Gnanadeepam, Dr. M. P. Sumathy, Dr. Dhanalakshmi, Dr. V. Shanthi, Dr. J. Jude and Dr. S. Jayalakshmi for their invaluable endeavour towards contribution to the diagnosis and preservation of vital source of information and materials to accomplish this study with ease.

Lastly, my deep appreciation to my parents, my in-laws, wife and daughter for their love, affection and encouragement.

Above all, I thank God for all the blessings.

INTRODUCTION

Ameloblastoma is a benign tumor of odontogenic epithelium which is thought to arise from enamel forming cells, particularly ameloblasts, but does not undergo differentiation to the point of enamel formation.[1] Its relative frequency equals the combined frequency of all other odontogenic tumors excluding odontomas. It is believed that they may arise either from rests of dental lamina, a developing enamel organ, epithelial lining of odontogenic cyst or from the basal cells of the oral mucosa.[1]

E-Cadherin is a calcium-dependent cell surface glycoprotein involved in diverse biological processes such as cell adhesion, cell recognition, control of cell division, differentiation, cell migration and morphogenesis.[2] Cytokeratin-14 and Cytokeratin-19 are type-I keratins (acidic nature), which form intermediate filaments that are usually expressed in simple epithelium.[6,7]

Various research studies have shown that E-Cadherin, Cytokeratin-14 and Cytokeratin-19 are highly sensitive and specific markers for ameloblastomas as well as other odontogenic lesions.[6,7,8] They have also found that these markers are differentially expressed in the various stages of ameloblasts in the developing tooth germ.

A study on E-Cadherin expression in the enamel organ of human tooth germ revealed, E-Cadherin was intensively expressed in IEE of cap, early and late bell stages but its expression decreased and progressively lost when IEE has differentiated to preameloblasts and secretory ameloblasts.[2] On contrary, a study on tooth germ of rat revealed, E-Cadherin expression was intense in the presecretory, transitional and reduced state ameloblasts but the expression was dramatically lower in secretory and matruative ameloblasts.[3] As there are no adequate studies of

E-Cadherin expression in human tooth germ, it would be prudent to study E-Cadherin expression in human tooth germ to correlate the expression pattern for comparison with ameloblastoma to ascertain its role in enamel formation.

Cytokeratin-14 is a differentiation marker utilized in the detection of presecretory ameloblasts of tooth germ[7]. Immunohistochemical studies in human tooth germ have shown strong Cytokeratin-14 positivity in inner enamel epithelium of human fetuses during early bell stages but the expression is gradually lost during late bell stages were inner enamel epithelium differentiated into secretory ameloblasts whereas, Cytokeratin-19 is intensively expressed (up-regulated) in secretory ameloblasts of late bell stages. Hence, Cytokeratin-14 and Cytokeratin-19 are reciprocal markers of differentiation of the cells of ameloblastic lineage.[7]

In ameloblastoma, various studies have shown E-Cadherin expression was evident in the peripheral columnar cells and intense in the central angular or polyhedral cells.[4] Cytokeratin-14 and Cytokeratin-19 were also expressed in the odontogenic islands of ameloblastoma but the information regarding the pattern of expression is not adequate.

Therefore, the purpose of this study is to evaluate the expression of E-Cadherin, Cytokeratin-14 and Cytokeratin-19 in tooth germ and ameloblastoma in order to determine morphological and functional characteristics.

AIMS AND OBJECTIVES

AIM:

The aim of this study is to evaluate the expression of E-cadherin, Cytokeratin-14 and Cytokeratin-19 in tooth germ and ameloblastoma.

OBJECTIVE:

The objective is to determine morphological and functional differentiation.

REVIEW OF LITERATURE

ODONTOGENESIS

Odontogenesis is complex embryological processes involving induction, differentiation and morphogenesis that results in development and eruption of the teeth. A series of sequential and reciprocal interactions between epithelial and ectomesenchymal cells (derived from neural crest cells) are fundamental in tooth development.[5] Odontogenesis initiates with the formation of a horseshoe shaped band of thickened oral epithelium in the future dental arches of upper and lower jaws known as primary epithelial band.[9] The cells of the primary epithelial band divide to form dental lamina and vestibular lamina due to change in orientation of their mitotic spindle and cleavage plane.[9] The continues and localized proliferative activity of anterior aspect of dental lamina, leads to the formation of epithelial down growths into the ectomesenchyme, at sites corresponding to the positions of the future deciduous teeth, from which enamel organ develops.[10] Enamel organ increases in size, sinks deeper into the ectomesenchyme and takes the shape of bud, cap and bell forming different stages of the tooth development.[9]

Bud stage: In the bud stage, the epithelial cells of dental lamina proliferate into the neural crest-derived ectomesenchyme forming a bud shaped structure.[9] The enamel organ consists of peripherally located low columnar cells and centrally located polygonal cells.[10] Ectomesenchymal cells adjacent to enamel organ begin to condense around the enamel organ.

Cap stage: During the cap stage, the epithelial cells of the enamel organ proliferate to form a cap on a ball of condensed ectomesenchymal cells, representing dental papilla.[5] This stage is characterized by proliferation, condensation of ectomesenchyme and osmotic imbibition of water into enamel organ.[9,10] The enamel organ contains cuboidal cells forming outer enamel epithelium, short columnar cells forming inner enamel epithelium and star shaped cells forming stellate reticulum. The enamel knot is a transient structure, formed by cluster of nondividing epithelial cells that act as a signaling center and reservoir to the dividing cells.[9,10]

Bell stage: Further growth during the proceedings stage results in deep invagination of the epithelium due to reciprocal pressure exerted by the dental papilla leading to formation of bell-like structure of the enamel organ.[9.10] This stage is characterized by histodifferentiation and morphodifferentiation.[9.10] The folding of enamel organ to form different crown shapes is due to differential rates of mitosis and differences in cell differentiation time.[9] The dental lamina joining the tooth germ to the oral epithelium is fragmented.[10] The enamel organ contains four different layers of epithelial cells that are cuboidal cells forming outer enamel epithelium, star shaped cells forming stellate reticulum, squamous cells forming stratum intermedium, and tall columnar cells forming inner enamel epithelium.[9] The cells of stratum intermedium show high activity of the enzyme alkaline phosphatase.[10] Dental papilla is enclosed in the invaginated portion of the enamel organ containing undifferentiated ectomesenchymal cells. The dental follicle is less distinct.[9]

Late bell stage: This stage is characterized by the commencement of mineralization and root formation.[10] Terminal differentiation of peripheral cell layer ectomesenchyme takes place due to reciprocal induction, forming dentin producing odontoblasts.[9] Similarly, inner enamel

epithelium differentiates to form ameloblasts producing enamel.[9] The inner enamel epithelium has organizing influence over the dental papilla, making the cells to differentiate. The formation of dentin occurs first as a layer along the future dentinoenamel junction in the region of future cusps and proceed pulpally and apically, followed by formation of enamel by ameloblasts over the dentin.[10] The cervical portions of the enamel organ gives rise to Hertwig's epithelial root sheath necessary for root formation. Dental follicle show circular arrangement of fibers resembling capsule, which later forms supporting structures of the tooth.[11]

STAGES OF AMELOBLAST DIFFERENTIATION

Tooth enamel differs from the other calcified tissues in being a product of epithelially-derived cells called ameloblasts.[11] Ameloblasts secrete matrix proteins and are responsible for creating and maintaining an extracellular environment favorable to mineral deposition.[9] This epithelial cell exhibits a unique life cycle characterized by phenotype changes that reflect its primary activity at various stages of enamel formation.[9] During the early bell stage, the cells present in the concave surface of enamel organ assume short columnar/cuboidal cells with centrally placed nuclei, situated opposite to undifferentiated ectomesenchymal cells of dental papilla, called inner enamel epithelium.[11,5]

At the stage of determination of crown shape, the cells of the inner enamel epithelium that are still cuboidal with centrally placed nuclei differentiate and lose their capacity to divide.[9] These cells begin a life cycle which will take them to preameloblast, presecretory ameloblast, secretory ameloblast, maturation ameloblast and finally protective stage ameloblasts immediately before cell death.[11]

In preameloblast stage, the cells become elongated to adopt a columnar shape (20µm) with nucleus moves towards the side closest to stratum intermedium and cytoplasm containing organelle.[11] There is an intact basement membrane separating the cells from dental papilla.[15] They secrete proteins containing high molecular weight amelogenin-like protein necessary for odontoblast differentiation and contribute to the bond strength between enamel and dentin.[3] This protein calcify later forming initial layer of structure-less enamel.

The preameloblast elongates further and differentiates into presecretory ameloblast forming the tallest cell of life cycle (40µm).[15] The basement membrane is lost and the cells are in direct contact with the dentin matrix.[1] There is no evidence of enamel formation.[5]

Eventually, the ameloblasts move outwards from the dentin matrix and develop a short conical process containing granules and vesicles, known as the Tomes' process. Now the ameloblasts are considered as secretory ameloblasts.[11] The secretory ameloblasts are columnar cells (25-40x5µm) arranged perpendicular to the forming dentin surface. They secrete enamel containing rods and inter rods (prismatic enamel).[11] The Tomes' process present in these cells interdigitates with newly formed enamel giving a picket-fence or saw-toothed appearance.[9]

As the enamel formation reaches full thickness, the morphology and function of ameloblasts changes. The cells now attain maturative stage in order to enhance mineralization by removing water and protein from the matrix and incorporation of calcium and phosphate for crystal growth.[11] The maturation ameloblasts loose Tomes' processes but their final secretory products are exocytosed from their flat surfaces to form a final layer of rodless enamel. [9] During the maturative phase the ameloblast undergo modulation between ruffle-ended and smooth ended surface.

Later, the maturative cells organize with stratum intermedium, stellate reticulum and outer enamel epithelium to form papillary layer. Finally, when enamel is fully mature, the ameloblast layer and the adjacent papillary layer regress and together constitute reduced enamel epithelium. The ameloblasts stops modulating, reduce their size, and assume a cuboidal appearance.[9] The reduced enamel epithelium protects the newly formed enamel from mesenchyme till the tooth erupts.[9]

AMELOBLASTOMA

Ameloblastoma is the true neoplasm of enamel organ type tissue which is does not undergo differentiation to the point of enamel formation. Robinson described this tumor that is 'usually unicentric, nonfunctional, intermittent in growth, anatomically benign and clinically persistent'.[18]

History:

Broca in 1868[18] was the first to report this lesion.

Malassez in 1885[19] coined the term 'adamantinoma'

Churchill in 1934[18] suggested the term 'ameloblastoma' to replace the term 'adamantinoma' as the later term implies formation of hard tissue, and no such material is present in this lesion.

Epidemiology:

Ameloblastoma is the second most frequently encountered tumor arising from odontogenic epithelium[26]. However, a study reported ameloblastoma to be the most common odontogenic

neoplasm in India.[27] The mean age of ameloblastoma is 35 years, with a range of 4-92 years.[19, 29] Men and Women are equally affected (47% females and 53% males).[19, 29] Environmental factors and individual patient variables such as general health and nutritional status modulate the incidence of the disease resulting, tumor to occur 10-15 years earlier in developing countries.[20]

Etiopathogenesis:

Ameloblastoma is thought to originate from sources that include residual tooth germ epithelium, epithelium of odontogenic cyst, stratified squamous epithelium, epithelium of the enamel organ and heterotrophic epithelium from pituitary gland.[21] Several authors describe possible pathogenic mechanisms that include nonspecific irritants such as extraction, caries, trauma, infection, inflammation, or tooth eruption, nutritional deficit disorders and viral pathogenesis.[30, 31] No evidence of HPV infection has been detected by morphological examination, immunohistochemistry, in situ hybridization and conventional PCR.[38]

WHO classification of ameloblastoma:

The WHO classification of ameloblastomas includes four types: unicystic, solid/multicystic, extraosseous/peripheral and desmoplastic.[37]

Unicystic ameloblastoma was first described in 1977 by Robinson and Martinez.[32] Clinically, unicystic ameloblastoma occurs in young patients, before the 5th decade of life with average age of 26 years,[33] and may behave less aggressive than multilocular lesions.[22] The tumor is characterized by lining epithelium that exhibits alterations described by Vickers and Gorlin.

Some cases show proliferative ameloblastic epithelium extending into lumen or islands of epithelium infiltrating into connective tissue wall.

Solid/multicystic ameloblastoma occurs in all areas of the jaws, rarely involves the sinonasal cavities and shows a marked preference for the posterior region of the mandible but this tumor has a site predilection for the symphysis in African children.[19,20] The histologic evaluation of these tumors is based on the cytological criteria produced by.[21] The tumors show two basic patterns, follicular and plexiform, which carry no clinical relevance.[37] However, some study showed follicular type has a high recurrence.[19] In many ameloblastomas, the proliferating epithelium exerts an inductive effect on the surrounding connective tissue stroma resulting in formation of zone of hyalinization immediately adjacent to epithelium.[20]

Desmoplastic ameloblastoma is most commonly found in the maxilla, in the anterior/premolar area and does not show the typical clinical and radiographic features of ameloblastoma. This tumor can be mistaken for non-ameloblastomatous lesions or even osteosarcoma.[34] Histologically, it has pronounced desmoplastic stroma with compressed tumor islands usually lacking central zone of stellate reticulum.[29]

Tumor islands often infiltrate into marrow spaces of surrounding bone and no capsule formation with high recurrence rate.

Peripheral ameloblastoma is defined as a tumor with histological characteristics of an intraosseous ameloblastoma but occurring in the soft tissue overlying the tooth-bearing regions of the maxilla and mandible.[35] Authors suggest that the tumor may arise from extraosseous epithelial remnants of dental lamina and its organ derivatives within the underlying connective tissue or from basal cell layer of the oral mucosa.[36, 23]

Histological subtypes:

Ameloblastoma is a polymorphic neoplasm consisting of proliferating odontogenic epithelium, which usually has a follicular or plexiform pattern, lying in a fibrous stroma. (WHO 2005).[28]

Various histologic patterns have been recognized but most tumor show a predominance of one pattern. The odontogenic epithelial component can be arranged in islands, cords and strands that may vary in size. The peripheral cells of the epithelial component are composed of tall columnar cells with hyperchromatic nuclei. The nuclei tend to be round to oval in shape with reversed polarity and palisading arrangement. Cytoplasm may show vacuoles. (Vicker and Gorlin 1970).[21]

- *Follicular ameloblastoma:* Composed of many small discrete islands of tumor cells with peripheral layer of cuboidal or columnar cells whose nuclei are generally polarized and central polyhedral cells resembling stellate reticulum. Cyst formation is relatively common in this type.
- *Plexiform ameloblastoma:* The tumor cells are arranged in irregular masses as a network of interconnecting strands. Each of these strands is bounded by a layer of columnar cells enclosing stellate reticulum-like cells. Cystic degeneration of the stroma can occur.

- *Acanthomatous ameloblastoma:* The tumor cells are arranged in discrete islands with the cells occupying the position of stellate reticulum undergo squamous metaplasia, occasionally with keratin formation.
- *Granular cell ameloblastoma:* Groups of lesional epithelial cells undergo granular cell transformation having abundant cytoplasm filled with eosinophilic granules that resemble lysosomes ultrastructually and histochemically.
- *Basal cell type of ameloblastoma:* Composed of nests of uniform basaloid cells resembling basal cell carcinoma. This is the rarest histologic subtype of ameloblastoma.
- *Desmoplastic ameloblastoma:* This variant is characterized by dense collagen stroma showing thin stands and cords of odontogenic epithelium. The epithelial proliferation almost seems to be compressed and fragmented by the dense hyalinized stroma.
- *Clear cell ameloblastoma, keratoameloblastoma, mucous cell differentiation in ameloblastoma and hemangiomatous ameloblastoma* are other variants.[24]

Cytokeratin-14 & Cytokeratin-19 expression in tooth germ and ameloblastoma

1. In 2013, **Pal SK[39]** et al. studied the immunohistochemical expression profiles of acidic epithelial keratins (CK-10, **CK13-20**) in **ameloblastomas** and **mouse tooth germs.** Their study included ameloblastomas (13 solid, 5 unicystic), 1 ameloblastic fibroma, 6 basal cell carcinomas and mouse tooth germs. The expression of CK-14 and CK-19 was found in both in the outer and inner cells of ameloblastoma. In the areas of stellate reticulum-like cells showing squamous metaplasia CK-19 was found in the squamous cells but CK-14 was

negative. In contrast to ameloblastoma, CK-14 was found to be expressed in the entire mouse tooth germ, but CK-19 expression was found only in the dental lamina.

2. In 2012, **Sudheer K**[43] et al. studied immunohistochemical analysis of dentigerous cyst and **ameloblastoma** using **CK-19, CK-14**, p53, p63 and Ki-67 in order to assess the differences in their nature and behavior. Five cases of ameloblastoma and five cases of dentigerous cyst were included in their study and were analyzed. They found that all the above mentioned markers stained both basal and suprabasal layers of ameloblastoma and dentigerous cyst with mean scores of each marker was found to be higher in ameloblastoma than dentigerous cyst. The expression of CK-14 and CK-19 were found to be intense in both peripheral columnar and central stellate cells of ameloblastoma.

3. In 2011 **Nel S**[5] et al. studied the immunohistochemical profile of odontogenic epithelium in **developing dog teeth** in order to find the usefulness of the markers to differentiate odontogenic from nonodontogenic epithelium. In their study 24 dog fetuses were grouped, sectioned and examined for **CK-14** and **CK-19**, amelogenin, p75 neurotrophin receptor and calretinin on different developmental stages of dog teeth. They considered Group-I as fetuses that had tooth germs in bud and cap stages of development with no ameloblast differentiation or dental hard tissue formation and Group-II as fetuses having bell stage of development with visible ameloblast differentiation and hard tissue formation. They found that the IEE of Group-I fetuses stained positive for both CK-14 and CK-19 with CK-14 being more intense. In group-II fetuses, the expression of CK-14 revealed focal staining in IEE, no staining in pre-secretory ameloblast, and intense staining in secretory ameloblast but

staining intensity decreases as enamel secretion continues. The expression of CK-19 in group-II fetuses revealed diffuse staining in IEE (except in cervical loop region), pre-secretory and secretory ameloblasts. The OEE of both group-I and group-II stained positive for CK-14, although their distribution and intensity varied. The dental laminae and cell rests of serres were also stained positive for both CK-14 and CK-19. The authors speculated that the decreased expression of CK-14 in pre-secretory ameloblast was necessary to disengage its anchorage and migrate for enamel matrix deposition.

4. In 2010, **Babu CN**[8] et al. studies the expression of Bcl2 (antiapoptotic protein), **Cytokeratin-14** and **Cytokeratin-19** in **ameloblastoma**. In their study 30 specimens of ameloblastomas were examined for expression of the above mentioned markers immunohistochemically. They found that Cytokeratin-14 and Cytokeratin-19 were expressed in both outer cells and stellate reticulum like cells of ameloblastomas. The expression of CK-14 was found in all 30 cases thereby giving 100% positivity but the expression of CK-19 was found only in 23 out of 30 cases thereby giving 76.6% positivity. The authors' interpreted that the intense expression of CK-14 in all ameloblastomas suggests that they may retain basal cell characteristics with potential for proliferation.

5. In 2009, **Hassan RM**[45] et al. studied morphological changes in the enamel and **Cytokeratin-14** distribution in **ameloblasts of diabetic rat mandibular incisor**. Forty five male albino rats were grouped, sectioned and examined the ameloblasts both histologically and with immunohistochemical expression of CK-14. The enamel surfaces were analyzed by scanning electron microscope. Control group consisting of 15 rats (6-7 months age) that received intraperitoneal injection of sterile saline only were considered group-I. Test group

consisting of 30 diabetic rats of similar age that received a single intraperitoneal injection of streptozotocin were considered as group-II. They found that CK-14 was expressed intensively in the ameloblasts of control group but its expression varied in diabetic group as some cases showed decreased staining.

6. In 2006, **Osman HI**[41] et al. studied the **CK-14** expression in various stages of developing **enamel organ of rat molars** using immunohistochemistry. Rat fetuses of embryonic day 10 to postnatal day 7 were included in their study. They found that the expression of CK-14 was intense in IEE and remnants of dental lamina of early bell stages, weak or not expressed in IEE in late bell stage, expressed in progenitor cells of cervical loop, and intensively expressed in preameloblasts and ameloblasts during initial enamel formation. The expression of CK-14 was also intense in stratum intermedium of late bell stage and progenitor cells within OEE and IEE of cervical loop region but weak in stellate reticulum of both early and late bell stages. The authors emphasized that CK-14 might have a role in preserving and supporting epithelial-mesenchymal interactions till early bell stage. With the formation of the first layer of dentin during the late bell stage, the epithelial-mesenchymal connectivity is lost. To achieve this ameloblast needs to lose CK-14 to fully disengage its anchorage. They concluded that developing enamel organ may be a suitable model for investigating cytokeratin expression and cell function.

7. In 2006, **Wato M**[47] et al. studied immunohistochemical expression of **cytokeratins** in **ameloblastoma**. In their study 31 solid ameloblastomas (13follicular and 18 plexiform) were examined for the expression of CK 1, 4, 6, 10, 10/13, 15, 16, 17, **19**, 20 and AE1/AE3. They

found that CK-19 was positive in all 31 cases of ameloblastomas. The expression CK-19 was found in stellate reticulum cells present in the inner portion of the tumor nests of follicular ameloblastoma. In plexiform ameloblastoma, some cells (both inner and outer layers) express CK-19. The authors concluded that, CK-19 is a marker of the odontogenic origin as it is expressed in all ameloblastomas.

8. In 2003, **Crivelini MM[40]** et al. studied cytokeratins in epithelia of odontogenic neoplasms. The purpose of their study was to describe the immunohistochemical expression of CK7, 8, 10, 13, **14**, 18, **19** and vimentin in epithelial components of the **human tooth germ** and **5 types of odontogenic tumors** in order to discuss histogenesis. The cytokeratins expressions mentioned above were analyzed in different stages of 3 human tooth germ (28^{th} to 32^{nd} week of gestation), 5 dental follicles with reduced enamel epithelium and 26 odontogenic tumors (10 Ameloblastomas, 4 AOTs, 4 CEOTs, 5 Ameloblastic fibromas and 3 Odontomas). They found that all the cells of enamel organ and dental lamina expressed CK-14, except in the secretory ameloblasts and in the portions of stellate reticulum/stratum intermedium containing advanced amelogenesis. The expression of CK-19 on tooth germ revealed positive staining in preameloblast, secretory ameloblast and part of OEE. In ameloblastomas, CK-14 was expressed in most tumoral cells, except part of the central stellate reticulum-like cells, outer columnar cells with vacuolated cytoplasm and metaplastic squamous cells. Whereas CK-19 was expressed in metaplastic squamous cells of cystic structures and central stellate reticulum-like cell. The authors suggested that loss of CK-14 expression in regions of advanced amelogenesis and positive CK-19 expression in preameloblast and secretory ameloblast were related to secretory differentiation. They also

hypothesized that CK-19 characterizes only ameloblasts and preameloblasts with complete differentiation, which does not occur in ameloblastomas, and the stimuli to a final differentiation process failed in the tumoral cells.

9. In 2002, **Fukumashi K**[42] et al. studied **cytokeratin expression** in different histological types of **ameloblastomas** and to discuss their histogenesis. In their study 73 cases of ameloblastomas (18 follicular, 43 plexiform, 6 acanthomatous, 3 granular type and 3 desmoplastic types) were examined for immunohistochemical expression of PK, KL1, CK8, **CK-19** and CK-13. They found that CK-19 was expressed in basal, supra basal cells and inner cells of all histologic types of ameloblastomas except in desmoplastic variant, which showed CK-19 positivity only in suprabasal and inner cells. They concluded that as these markers were found in the odontogenic epithelium, ameloblastoma may arise from odontogenic epithelium in the prenatal period rather than basal layer of oral mucosa.

10. In 2001, **Kumamoto H**[6] et al. studied the expression of amelogenin and **Cytokeratin-19** in **epithelial odontogenic tumors**. In their study 33 ameloblastomas, 3 CEOT, 2 clear cell odontogenic tumors and 5 malignant ameloblastomas were included. They found that CK-19 was diffusely expressed in neoplastic cells of ameloblastomas and decreased expression was found in keratinizing cells of acanthomatous variants.

11. In 2000, **Domingues MG**[7] et al. studied expression of cytokeratins in human tooth germ (5-7 months gestational age) corresponding to bell stage to ascertain stage specific differentiation process in enamel organ. They analyzed CK 7, 8, 10, 13, **14**, 16, 17, 18 and **19**

on the tooth germ obtained from **7 human fetuses**. They found that the expression patterns of CK-14 and CK-19 showed differences related to stage specific differentiation. The expression of CK-14 was intense in the IEE at early bell stage and down-regulated in late bell stage where IEE differentiated into ameloblasts. On the contrary, the expression of CK-19 was weak in IEE at early bell stage and later showed intense positivity in fully differentiated ameloblasts during late bell stage. The expressions of both CK-14 and CK-19 were also found in stellate reticulum, OEE and remnants of dental lamina with intense positivity of CK-14 at early bell stages. The authors interpreted that in the process of conversion of IEE to ameloblast, CK-14 and CK-19 presented opposite expression patterns with down-regulation of CK-14 and up-regulation of CK-19. They suggested CK-14 as an anchorage protein which is necessary for epithelial-mesenchymal interactions in early bell stage and needs to be down-regulated in fully differentiated ameloblast to disengage and gain space for enamel matrix formation. They concluded that CK-19 was the marker for ameloblast differentiation.

12. In 1999, **Ong'uti MN**[44] et al. immunohistochemically studied cytokeratin expression in **ameloblastomas** of Kenyan population. The aim of their study was to assess expression of cytokeratins in ameloblastomas and to correlate with their clinical and histological features. Thirty nine ameloblastomas were studied for the expression of CK-1, CK-5, CK-6, CK-7, CK-8, CK-10, **CK-14**, CK-16, CK-18 and **CK-19**. They found all the cases stained positive for both CK-14 and CK-19 in basal, suprabasal and central stellate reticulum-like cells. The authors suggested that the expression of CK-14 and CK-19 in ameloblastoma may retain basal cell characteristic with a potential for proliferation.

13. In 1989, **Hikinheimo K**[46] et al. studied patterns of expression of intermediate filaments in **ameloblastoma** and **human fetal tooth germ**. They found that **Cytokeratin-19** was expressed in epithelial elements of ameloblastomas and tooth germ. They concluded that by considering the pattern of cytokeratins expression, ameloblastomas originate from odontogenic epithelium and not a direct derivative of basal cells of oral epithelium.

14. In 1989, **Luo W**[48] et al. analyzed cytokeratin gene expression in human **ameloblastoma** using in situ hybridization technique. Complementary DNA clones corresponding to **CK-14** and CK-10 were used as probes for analysis. They found that CK-14 gene transcript was present within the epithelial cells of ameloblastomas. In an atypical infiltrating ameloblastoma CK-14 transcript could not be identified.

15. In 1989, **Kasper M**[49] et al. studied the distribution of intermediate-filament proteins in **human tooth germ.** They examined expression of CK 5, 7, 8, **14**, 17, 18, **19** on fetal oral epithelium and human enamel organ by immunohistochemistry and gel electrophoresis. They found that CK-14 and CK-19 were expressed in the enamel organ of human tooth germ.

E-Cadherin expression in tooth germ and ameloblastoma

1. In 2013, **Mello**[56] et al. studied the relationship of CD1a-positive Langerhans cells with **E-Cadherin** in **ameloblastomas** and keratocystic odontogenic tumors. Thirty eight cases of odontogenic tumors (10 solid ameloblastomas, 10 unicystic ameloblastomas and 18 KCOTs) were immunohistochemically examined for CD1a and E-Cadherin. They found that E-Cadherin was expressed in cytoplasm and membrane of stellate reticulum-like cells present in the central region of solid ameloblastoma tumor nests. In unicystic ameloblastoma, E-

Cadherin was expressed in suprabasal layers of epithelial lining. The authors believe that decreased E-Cadherin expression in ameloblastoma, when compared to KCOTs, support the local invasive pattern.

2. In 2012, **Florescu A**[53] et al. studied immunohistochemical expression of MMP-9, TIMP-2, **E-Cadherin** and vimentin in **ameloblastomas** in order to know the mechanisms involved for their aggressive behavior. Seventeen cases of solid ameloblastoma (12 follicular, 3 acanthomatous and 2 granular cell) were included in the study. They found that immunoreactivity of E-Cadherin was restricted to the neoplastic epithelial component of all ameloblastomas. The stellate reticulum-like cells and central angular or polyhedral cells stain intensively whereas outer columnar cells stain weakly (especially to the invasive front). The expression of E-Cadherin was also weak/ lost in the areas that show squamous metaplasia. The pattern of E-Cadherin reactivity was predominantly on the membrane at cell-cell boundaries, but cytoplasmic reaction was also noticed. They concluded that the expression of these markers may serve as an indicator for the degree of local aggressiveness of ameloblastomas.

3. In 2010, **Gonzalez-Alva P**[54] et al. studied immunohistochemical expression of podoplanin, **E-Cadherin**, and vimentin in **ameloblastomas** in order to evaluate tumor progression and to investigate epithelial-mesenchymal transition. Thirty eight cases of ameloblastomas (9 follicular, 19 plexiform, 7 acanthomatous and 3 unicystic) were included in the study. They found that in solid/multicystic ameloblastomas, the expression of

E-Cadherin was evident on membrane at cell-cell boundaries of central angular or stellate reticulum-like cells and its expression was less at cell-cell boundaries and cytoplasm of outer columnar cells. In unicystic ameloblastomas, the expression of E-Cadherin was intense in upper layers of cystic lining, and decreased expression in basal cell layer. E-Cadherin expression was also found to be weak or lost in keratinizing areas of acanthomatous ameloblastomas. Moreover, they found some tumor nests within large ameloblastomas that had lost E-Cadherin expression.

4. In 2008, **Alves P**[51] et al. studied immunohistochemical expression of **E-Cadherin** and β-catenin in **ameloblastomas** and human **tooth germ** in order to determine their roles in cell differentiation and behavior of the tumor. In their study 21 cases of ameloblastomas (16 solid and 5 unicystic) and 5 human tooth germs in the bell stage were immunostained for expression of E-Cadherin and β-catenin. They found that all the cases of ameloblastoma and tooth germs were immunopositive for E-Cadherin. In solid and unicystic ameloblastomas, E-Cadherin was expressed in the cell membrane and cytoplasm of outer columnar cells and loosely organized inner cells. In tooth germ (bell stage), E-Cadherin was expressed in cell membrane and cytoplasm of enamel organ cells, with more intense in the cell membrane of stellate reticulum and weak in IEE, OEE and stratum intermedium. They concluded that expression of E-Cadherin may be related to the process of cell differentiation.

5. In 2007, **Chen M**[58] et al. studied expression of TGF-β1 and **E-Cadherin** in **human tooth germ** development in order to know their significance. 36 human fetuses of gestational age 8-34 weeks were included in their study. They found that during bud and cap stages,

E-Cadherin was expressed in enamel organ and dental lamina. During early bell stage, E-Cadherin expression was positive in ameloblast and OEE. During late bell stage, the expression of E-Cadherin was strongly positive in ameloblast, odontoblast which neared hard tissue along with positivity in OEE and dental papilla. They concluded that expression of E-Cadherin had close correlation with development, secretion and mineralization of enamel and dentin.

6. In 2002, **Heymann R[50]** et al. studied the distribution of **E- and N- Cadherin** in **developing and functional human teeth** under normal and pathological conditions. In their study five human fetuses (8 to 30 gestational weeks), permanent teeth (both intact and carious) scheduled for extraction, cell cultures from dental pulp and periodontal ligament were immunohistochemically examined. They found that E-Cadherin was expressed in the cap and bell stages of developing human tooth germ. In the cap stage, expression of E-Cadherin was intense in the enamel organ. During early and late bell stage, the expression of E-Cadherin was found in proliferating inner enamel epithelium (cervical loop area) but its expression decreased and progressively lost in the areas where IEE has acquired a higher degree of differentiation (pre-ameloblasts and ameloblasts). E-Cadherin also stained outer-enamel epithelium and stellate reticulum in cap and bell stages but its expression was weak during late bell stages. The authors believe the expression pattern of E-Cadherin on developing tooth germ showed inverse gradient when compared to oral epithelium, where E-Cadherin was expressed in the non-proliferating (differentiated) cells suggest that molecular mechanism controlling the tooth and the oral epithelium differ.

7. In 2000, **Sorkin C**[55] et al. studied immunohistochemical expression of **E-Cadherin**, α, β, γ catenins and $p120^{ctn}$ in **developing tooth germ (late-bell stage)** during amelogenesis of **rat** incisor in order to ascertain their roles on cell mobility, adhesion and differentiation. They found that E-Cadherin expression was strong in the presecretory, transitional, and reduced stage ameloblasts but was dramatically lower in the secretory and maturation stage ameloblasts. Although diffuse staining was observe throughout the cytoplasm of presecretory, transitional and reduced stage ameloblasts, staining was concentrated along basolateral cell membranes. The expression of E-Cadherin was intense in the stratum intermedium of presecretory and secretory stages but decreased expression was found in papillary cell layer during maturation and reduced stages.

8. In 1999, **Kumamoto H**[4] et al. studied immunoexpression of **E-Cadherin** and α-catenin in **epithelial odontogenic tumors** and **tooth germ** to clarify the possible role cell adhesion in oncogenesis and cytodifferentiation. Twenty-nine cases of epithelial odontogenic tumors (24 ameloblastomas, 2 calcifying odontogenic tumors, 1 clear cell odontogenic tumor and 2 malignant ameloblastomas) were studied for expression of E-Cadherin and α-catenin. The results were compared with the results obtained immunostaining of 12 human tooth germ (late-bell stage) cases. They found that E-Cadherin was strongly expressed on the surfaces of stellate reticulum and stratum intermedium, and slightly expressed on the cell-cell boundaries of IEE, OEE and dental lamina. Similarly, E-Cadherin expression in the follicular and plexiform ameloblastomas were detected strongly on cell surfaces of central angular or polyhedral, and slightly on cell-cell boundaries of outer columnar or cuboidal cells.

9. In 1995, **Palacios J**[52] et al. studied the expression of **E- and P-Cadherin** in different stages of developing **mouse tooth germ**. Mouse fetuses of embryonic days 13-19 were included in their study. They found that during bud stage all the epithelial cells of presumptive enamel organ expressed E-Cadherin. During cap and early bell stages, E-Cadherin was strongly expressed in the cells of the dental lamina, outer enamel epithelium, stellate reticulum and stratum intermedium but in the cells of inner enamel epithelium its expression pattern changed dynamically with development. The expression of E-Cadherin in IEE during early bell stage was mainly present in cervical loop region. In the late bell stage, proliferating epithelial cells in cervical loop region continued to express E-Cadherin. Polarizing pre-ameloblasts express E-Cadherin all over the surface but polarized secretory ameloblasts express only on their basal and apical poles, where zonula adherens type of cell-cell junctions was located.

MATERIALS AND METHODS

1. In this study five human fetuses (dead or aborted) of 20-24 gestational weeks were obtained from the Institute of Obstetrics and Gynecology (IOG), Madras Medical College, Egmore, Chennai-600008, India, after getting the due ethical clearance. The gestational ages were estimated by measuring the length of the fetus.
2. The unclaimed dead/aborted fetuses that will normally be discarded by the IOG were only included in the study, for which written or oral permission is not required from the concerned parents.
3. The procedures for handling and using the fetuses for research purpose were followed according to Indian Council of Medical Research (ICMR) guidelines 2006[60] as shown in the **Table-I**.

Table-I: Shows ICMR guidelines for using fetal tissues

- Termination of pregnancy should not be sought with a view to donate fetal tissue in return for possible financial or therapeutic benefits.
- Consent to have a termination of pregnancy and donation of fetal material for purpose of research or therapy should be taken separately.
- The medical person responsible for the care of the pregnant woman planning to undergo termination of pregnancy and the person who will be using the fetal material should not be the same. The women shall not have the option to specify the use for a particular person or in a particular way.
- The identity of the donor and the recipient should be kept confidential.

4. In this study, the exclusion criteria for the fetal tissue were (a) -dead/aborted fetuses that are claimed by the parents, (b) -live but aborted fetuses, and (c) -fetuses used for the purposes of transplantation.

5. The selected fetuses were carefully dissected and their maxillary and mandibular processes were fixed in 10% neutral buffered formalin, decalcified with 50% HCL for one hour and processed.
6. The anterior segments of the maxillary and the mandibular processes were sectioned to study the late bell stages. The posterior segments of maxillary and mandibular processes were sectioned to study both early and late bell stages.
7. A total of 12 tooth germs from 5 human fetuses were included in this study. Out of the 12 tooth germs, serial sections from 8 tooth germs (6 showing late bell stage and 2 showing early bell stages) were obtained.
8. The tooth germs were histologically assessed with reference to the different stages of ameloblasts differentiation in the routine H&E sections.
9. The differentiation stages of ameloblasts [inner enamel epithelium as in early bell stage (IEE-EBS), inner enamel epithelium as in late bell stage (IEE-LBS), preameloblasts as in early bell stage (PA-EBS), preameloblasts as in late bell stage (PA-LBS), presecretory ameloblasts (PA) and secretory ameloblasts (SA)] are described as in the **Chart-I.**
10. Each ameloblasts of the tooth germ were categorized as follows in the **Table-II**.

Table-II: categorizing tooth germ

Group-Ia	Inner enamel epithelium from both early and late bell stages (IEE).
Group-Ib	Preameloblast as in early bell stages (PA-EBS).
Group-Ic	Preameloblasts as in late bell stages (PA-LBS).
Group-Id	Presecretory ameloblasts (PSA).
Group-Ie	Secretory ameloblasts (SA).

11. A total of 30 cases of ameloblastoma were retrieved from the archival samples of the Department of Oral Pathology, Tamil Nadu Government Dental College and Hospital (TNGDC & H), Chennai-600003, India. From which, ten cases of ameloblastomas that displayed granular cell changes, desmoplasia and non-specific lining were excluded.

12. Thus, the final sample comprised of 20 cases of ameloblastoma (12 solid and 8 cystic). From these 20 cases, multiple regions were evaluated with H&E sections to assess the level of differentiation of the cellular elements especially the outer cells of the tumor follicles or strands. A total of 31 regions with more than one region were selected in a case on the basis of the outer cell morphology. However, not more than 4 regions were selected in any given case.
13. The selected regions were further categorized depending on the outer cell morphology into cells resembling as inner enamel epithelium (IEE), preameloblasts as in early bell stage (PA-EBS), preameloblasts as in late bell stage (PA-LBS), presecretory ameloblasts (PSA), secretory ameloblasts (SA) and unclassifiable on the basis of comparable cell types in the enamel organ of the tooth germ. Out of 31 regions initially assessed, 5 regions were excluded in the study as their cell morphology appeared atypical with reference to the cellular features of the tooth germ **(Fig-57 and Fig-58).** Finally, 26 regions were taken into consideration for evaluation in this study and were grouped as shown in **Table-III**.

Table-III: shows various groups and clinical cell types

Group-IIa	Regions that show outer cell morphology similar to IEE.
Group-IIb	Regions that show outer cell morphology similar to (PA-EBS).
Group-IIc	Regions that show outer cell morphology similar to (PA-LBS).
Group-IId	Regions that show outer cell morphology similar to (PSA).
Group-IIe	Regions that show outer cell morphology similar to (SA).

14. After assessment of the individual regions and cell types comparable to the tooth germ, immunohistochemical study was performed using pre-diluted markers to study both tooth germ and ameloblastoma. The markers are shown the **Table-IV.**

Table-IV: Markers used for evaluation of tooth germ and ameloblastoma

Cytokeratin-14	**Cytokeratin-19**	**E-Cadherin**
1:100 dilutions, Mouse monoclonal-clone LL002, from PathnSitu Biotech Laboratories, Livermore, CA, USA.	1:100 dilutions, Rabbit monoclonal-clone EP72, from PathnSitu Biotech Laboratories, Livermore, CA, USA.	1:100 dilutions, Rabbit monoclonal-clone EP6, from PathnSitu Biotech Laboratories, Livermore, CA, USA.

15. Sections of 3µm for H&E and immunohistochemistry were made.
16. The normal oral epithelium was used as a positive control for CK-14, CK-19 and E-Cadherin. The negative controls were employed by replacing primary antibody with Tris-buffered saline (TBS).
17. Immunohistochemical evaluation was done in tooth germs and ameloblastomas based on observation of brown end-product at the site of target antigen with a light microscope.
18. The expression pattern of CK-14, CK-19 and E-Cadherin were carefully evaluated among different groups both in the tooth germs and ameloblastomas.
19. The results were compared and illustrated.

Procedure for Hematoxylin and Eosin staining:[58]

- The sections were de-paraffinized in two changes of fresh xylene for 10 minutes each, re-hydrated with descending grades of alcohol.
- The sections were drained and stained regressively with hematoxylin for 10 minutes.
- The slides were drained and washed with running water until the sections are blue or 5 minutes (whichever is less).
- The sections were differentiated with 1% acid alcohol (1%HCl in 70% alcohol) for 5 seconds and washed in tap water for 5 to 10 minutes.
- Bluing of the sections was done by dipping the slides in alkaline solution (ammonia water) for 5 minutes.
- The sections were counterstained with 1% eosin Y for 2 minutes.
- The sections were washed in running water for 3-4 minutes, to differentiate the eosin stain.
- After draining, the sections were dehydrated in ascending grades of alcohol.
- The sections were cleared with 2 changes of xylene 30 seconds each.
- After clearing, the sections were mounted with Distrene 80 Dibutyl phthalate Xylol (DPX).
- The stained and mounted slides were examined under light microscope to confirm the histopathologic parameters.

Procedure for immunohistochemistry:[58,59]

- The sections of 3µm were made in 3-aminopropyltriethoxysilane (APES) coated slides.
- The sections were de-paraffinized in three changes of fresh xylene 5 minutes each.

- The sections were hydrated with two changes of absolute alcohol for 5 minutes each, followed by 90%, 70% and 50% graded alcohol 5 minutes each respectively.
- The sections were washed in 3 changes of distilled water, 2 minutes each.
- Heat induced epitope retrieval was performed using Tris-EDTA buffer, of pH-9.0 as per the guidelines of the manufacturer.
- Both Tris-EDTA (retrieval buffer) and Tris-buffered saline (wash buffer) were prepared in the laboratory, Department of Oral Pathology, TNGDC & H, Chennai. The chemicals needed for the buffer preparation were purchased from Microfine laboratories Chennai, India.
- Antigen retrieval was performed by pressure cooker method. In this method, 500ml of Tris-EDTA buffer was heated with the slides in the pressure cooker for 15-20 minutes or 3-4 whistles whichever is less.
- The slides were removed after bench cooling, washed in distilled water 3 changes, 2 minutes each.
- The wash buffer used was 0.5M Tris-buffered saline titrated to pH 7.4 to 7.5.
- Endogenous peroxide activity was blocked by incubating the sections in 3% hydrogen peroxide for 5 minutes at room temperature.
- The sections were rinsed with TBS wash buffer 3 changes, 2 minutes each.
- The sections were incubated with pre-diluted primary antibodies for 45 minutes in a moist chamber at room temperature.
- Antibodies used were Cytokeratin-14 (1:100 dilution, mouse monoclonal-clone LL002 from PathnSitu Biotech Laboratories, Livermore, CA, USA), Cytokeratin-19 (1:100 dilution, rabbit monoclonal-clone EP72 from PathnSitu Biotech Laboratories, Livermore,

CA. USA) and E-Cadherin (1:100 dilution, rabbit monoclonal-clone EP6 from PathnSitu Biotech Laboratories, Livermore, CA, USA).

- The sections were rinsed with TBS wash buffer 3 changes, 2 minutes each.
- The sections were kept in Polyexcel target binder reagent for 15 minutes, followed by rinsing with 3 changes of TBS wash buffer 2 minutes each.
- The sections were then incubated with Polyexcel HRP (secondary antibody from PathnSitu Biotech Laboratories) for 15 minutes and rinsed with TBS wash buffer.
- The antigen complexes were visualized by incubating the sections in DAB (3,3'-diaminobenzidine) substrate-chromogen.
- The sections were finally washed and counter stained with Harris's hematoxylin.
- The sections were dehydrated with graded alcohol, cleared with xylene and mounted with Distrene 80 Dibutyl phthalate Xylol (DPX).
- Positive controls for Cytokeratin-14, Cytokeratin-19 and E-Cadherin were used as indicated by the manufacturer.
- Negative controls were performed by replacing the primary antibody with TBS.

Evaluation of slides:

- The stained slides were evaluated by two pathologists using a binocular research microscope (Olympus BX43) under 2x, 4x, 10x, 40x objectives.
- The immunohistochemical evaluation was based on observation of brown end-product at the site of target antigen.
- The sections stained with Cytokeratin-14, Cytokeratin-19 and E-Cadherin was evaluated using descriptive and semiquantitative methods.

- The scores were made depending on the visual assessment in the light microscopy as described in **Table-V.**

Table-V: scoring criteria

N	Negative
P+	Mild staining
P++	Intense staining
N=P	Doubtful staining
P+(C)	Mild cytoplasmic staining
P+(M)	Mild membranous staining
P++(C)	Intense cytoplasmic staining
P++ (M)	Intense membranous staining

- Statistical analysis was performed using Pearson Chi-Square test through SPSS software.

RESULTS

The morphological assessment of tooth germ and ameloblastoma are as follows

Assessment of the tooth germ

Early bell stage:

The enamel organ assumes a bell shape. The dental lamina joining the tooth germ to the oral epithelium is fragmented. It is possible to recognize the shape of the crown pattern to some extent. Four different cells are recognized in the enamel organ, they are inner enamel epithelium, stratum intermedium, stellate reticulum and outer enamel epithelium. The cells of inner enamel epithelium are columnar shaped and show gradual differentiation from the cervical loop to the cusp tips to form preameloblasts (PA-EBS) (explained in **CHART-I**). Stratum intermedium cells are found adjacent to the areas where inner enamel epithelium shows differentiation to preameloblasts (PA-EBS). The stellate reticulum cells are star shaped with long processes that anastomose each other. The layer of outer enamel epithelium contains cuboidal to short columnar shaped cells with indentations. Dental papilla is enclosed in the invaginated portion of the enamel organ,which contains undifferentiated ectomesenchymal cells. The basement membrane separating enamel organ from dental papilla is distinct. The outer layer of dental papilla shows condensation of cells especially adjacent to preameloblasts (PA-EBS). The dental follicle is less distinct.

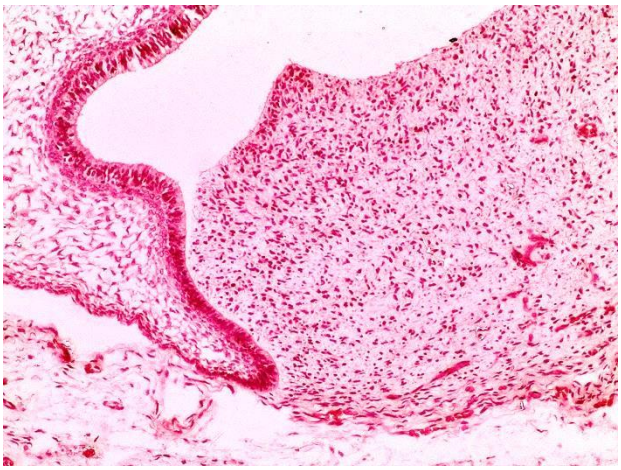
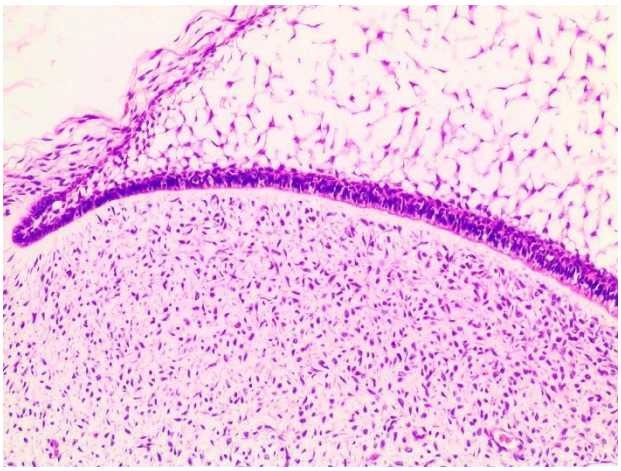
Late bell stage:

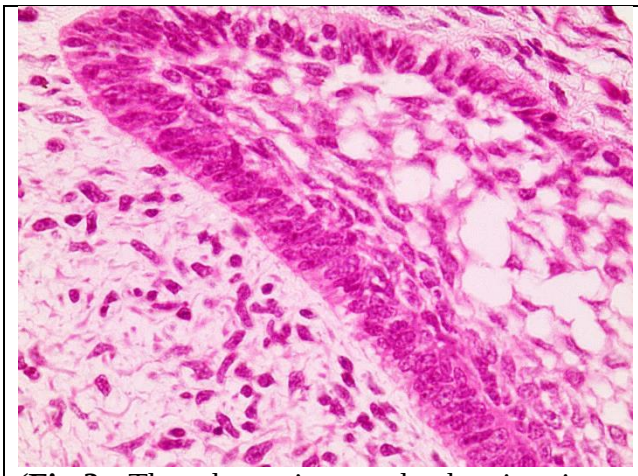
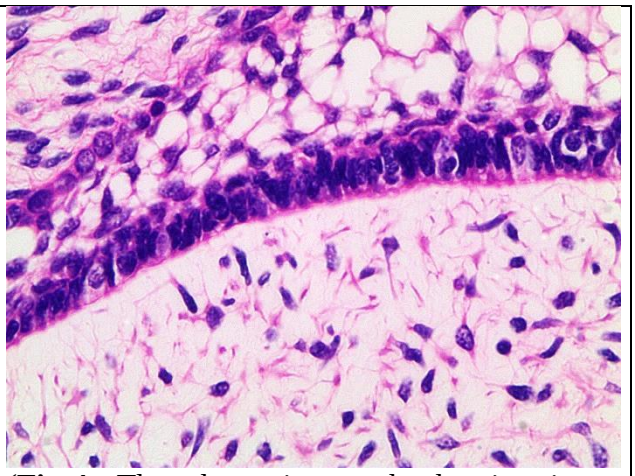
This stage is characterized by commencement of mineralization (dentin and enamel). The dental lamina joining the tooth to oral epithelium is fragmented. The shape of the crown pattern can be recognized. The inner enamel epithelium show gradual differentiation from cervical loop

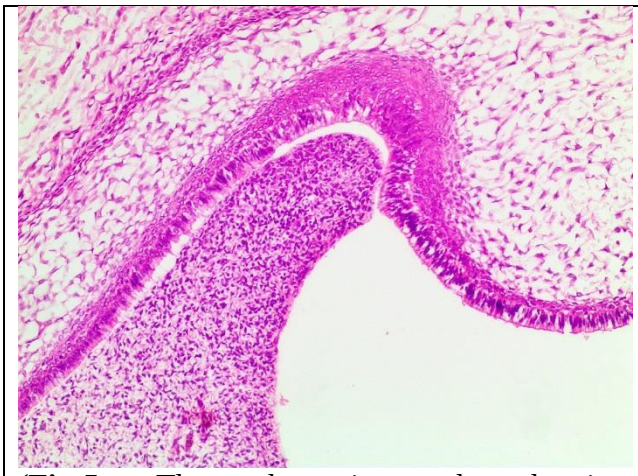
to the cusp tips forming preameloblasts (PA-LBS), presecretory ameloblasts (PSA) then to secretory ameloblasts (SA)(explained in **CHART-I**). The stratum intermedium cells are squamous shaped cells that are found in the areas adjacent to preameloblasts, presecretory ameloblasts (more prominent) and secretory ameloblasts. The stellate reticulum cells are star shaped with long processes that anastomose each other. The stellate reticulum starts showing decrease in thickness as the enamel formation continues. The layer of outer enamel epithelium contains cuboidal to short columnar shaped cells with indentations. The ectomesenchymal cells of dental papilla show differentiation to odontoblasts forming dentin. The dental sac is not an apparent structure.

CHART-I

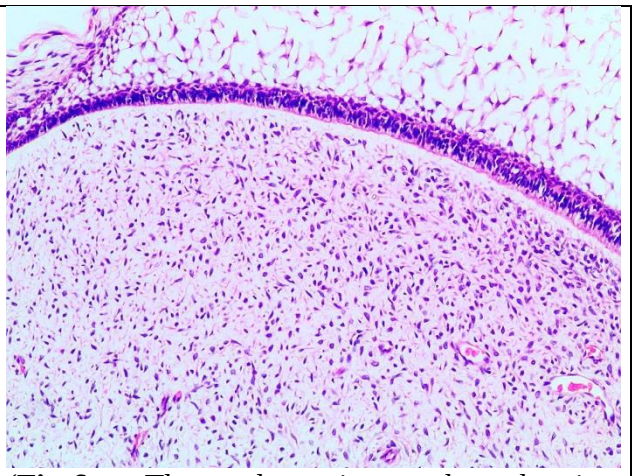
Showing morphology of different types of ameloblasts in the developing tooth germ.

Inner enamel epithelium (Early bell stage): The zone of inner enamel epithelial cells extends from the tip of the cervical loop to the point of transition of preameloblasts. These cells are columnar shaped cells containing round to oval shaped nucleus, which is arranged at different levels (predominantly central zone) and occupies almost the entire cell (**Fig-3**). A distinct basement membrane zone is visible. The cells are bordered by undifferentiated ectomesenchymal cells of the dental papilla on the one side and stellate reticulum cells on the other side.	**Inner enamel epithelium (Late bell stage)**: The zone of inner enamel epithelial cells extends from the tip of the cervical loop to the point of transition of preameloblasts. These cells are columnar shaped cells containing round to oval shaped nucleus, which is arranged at different levels (predominantly central zone) and occupies almost the entire cell (**Fig-4**). A distinct basement membrane zone is visible. The cells are bordered by undifferentiated ectomesenchymal cells of the dental papilla on one side and stellate reticulum cells on the other side with an acellular but fibrillar zone is interposed between them.
 (**Fig-1:** The photomicrograph showing inner enamel epithelium of early bell stage (x100, H&E).	 (**Fig-2:** The photomicrograph showing inner enamel epithelium of late bell stage (x100, H&E).

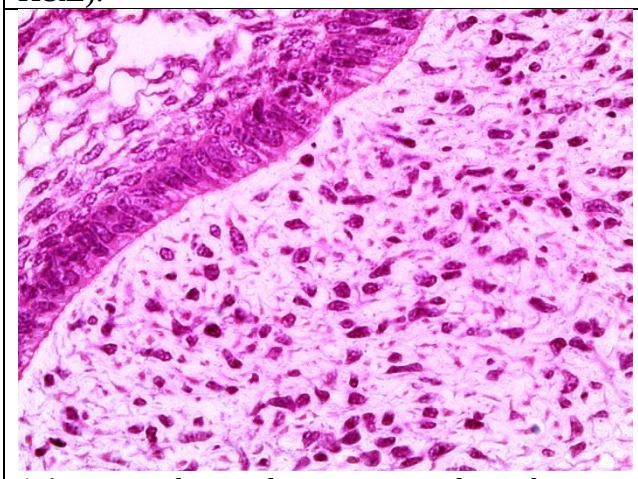
 (**Fig-3:** The photomicrograph showing inner enamel epithelium of early bell stage (x400, H&E). The nuclei occupying almost entire cell.	 (**Fig-4:** The photomicrograph showing inner enamel epithelium of late bell stage (x400, H&E). The nuclei occupying almost entire cell.
Preameloblasts (Early bell stage): The zone of preameloblast cells extends from the transition of inner enamel epithelium to the slopes of the cusp tip. It shows gradual differentiation and the cells are tall columnar shaped cells containing oval to long and slender nucleus especially at the cuspal slopes. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell. The cells are bordered by undifferentiated but condensed ectomesenchymal cells of the dental papilla on one side and stratum intermedium cells on the other side. The acellular zone between them is less distinct. **Note:** it was not possible to differentiate between stellate reticulum and stratum intermedium in all the examined tissues. However, the continuity and condensation of the cells at the cusp tip imparts the appearance of stratum intermedium (**Fig-5**). **Note:** the preameloblasts of early bell stage is slightly taller than preameloblasts of late bell stage especially at the cusp tip (**Fig-9**).	**Preameloblasts (Late bell stage):** The zone of preameloblast cells extends from the transition point of inner enamel epithelium to the transition point of presecretory ameloblasts. The cells are tall columnar shaped cells containing oval shaped, elongated, hyperchromatic nucleus. The nucleus shows reversed polarity with apparent pseudostratification (but no overt palisading) and occupies almost half of the cell. The cells are bordered by undifferentiated but condensed ectomesenchymal cells of the dental papilla on one side and stratum intermedium cells on the other side. There is no evidence of hard tissue formation and an acellular zone intervenes between the preameloblast layer and dental papilla with an intact basement membrane. **Note:** compared to the late bell stage, the preameloblasts of early bell stage are slightly taller especially at the cusp tip (**Fig-10**).



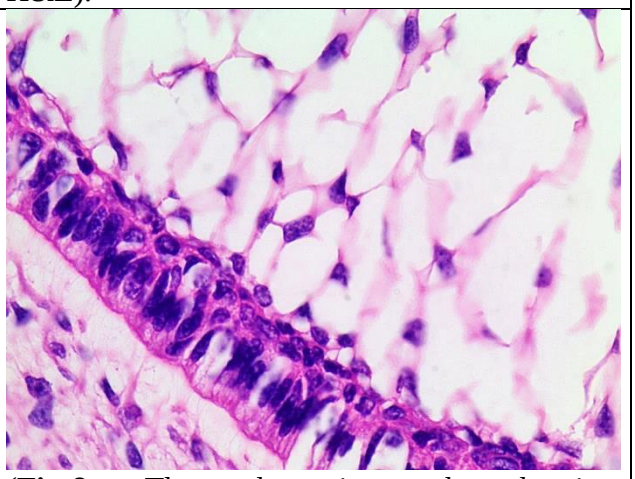
(**Fig-5:** The photomicrograph showing preameloblasts as in early bell stage (100x H&E).



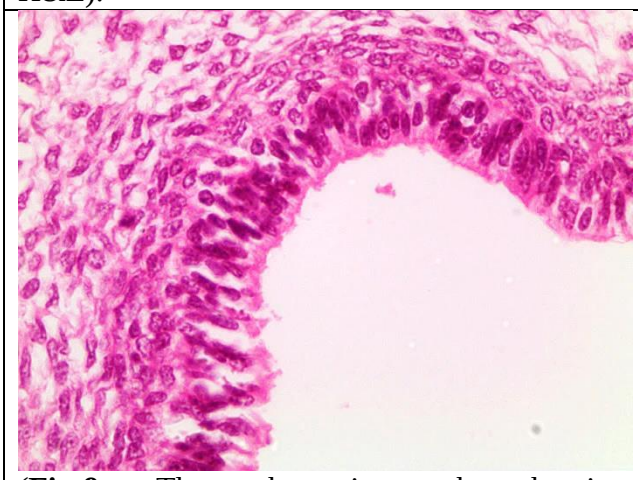
(**Fig-6:** The photomicrograph showing preameloblasts as in late bell stage (100x H&E).



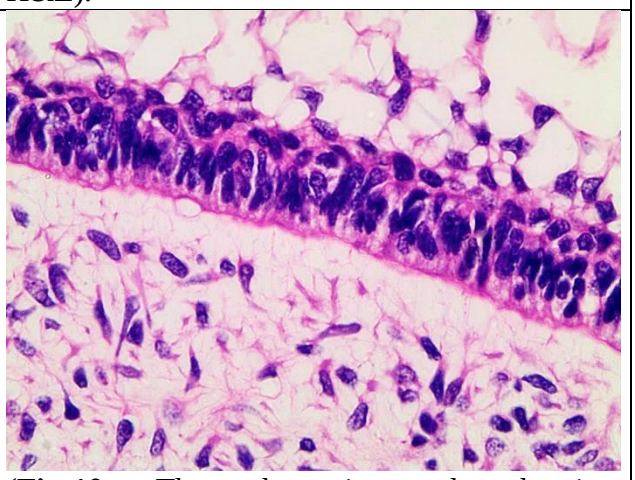
(**Fig-7:** The photomicrograph showing preameloblasts as in early bell stage (400x H&E).



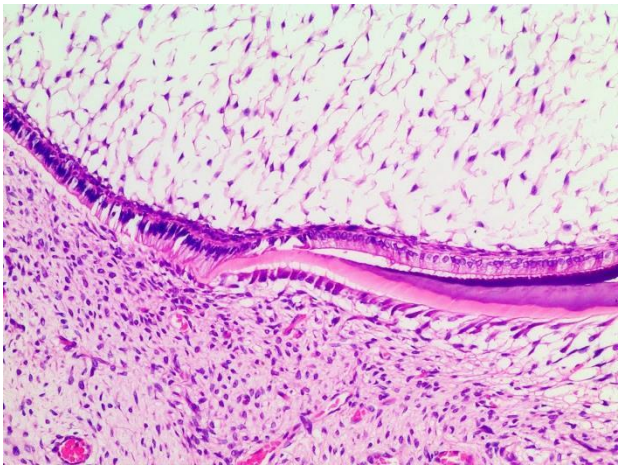
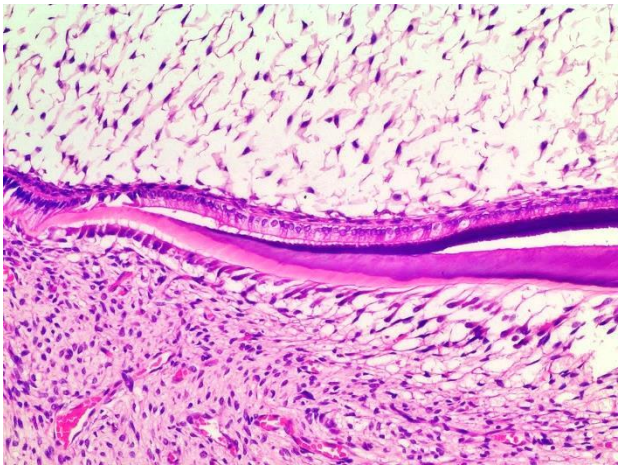
(**Fig-8:** The photomicrograph showing preameloblasts as in late bell stage (400x H&E).

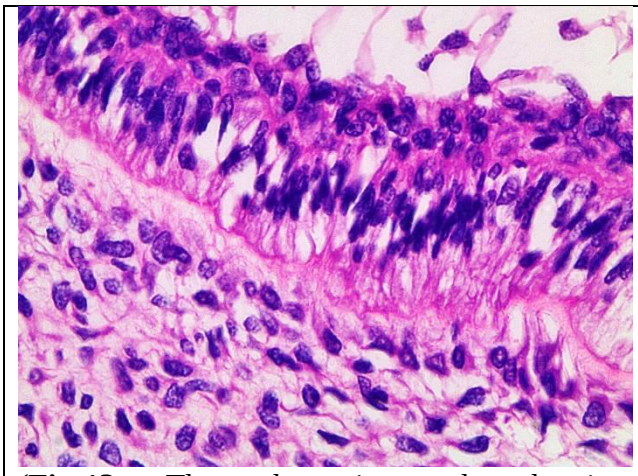


(**Fig-9:** The photomicrograph showing preameloblasts as in early bell stage (400x H&E).



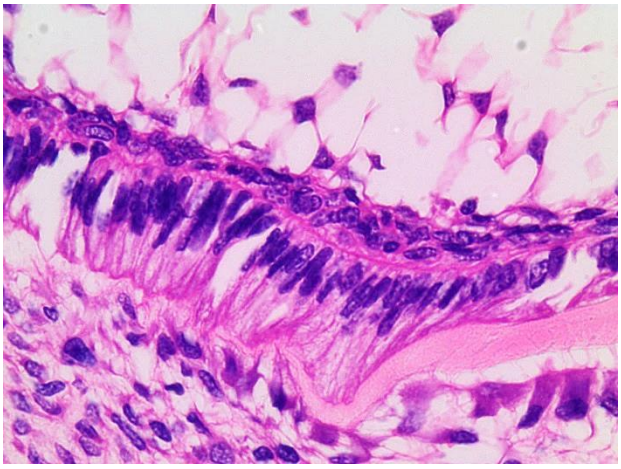
(**Fig-10:** The photomicrograph showing preameloblasts as in late bell stage (400x H&E).

Presecretory ameloblasts (Late bell stage): The zone of presecretory ameloblasts is a short segment of cells that extends from the transition point of preameloblasts to transition point of secretory ameloblasts. These cells are more tall columnar shaped cells containing long and slender nucleus with increased cytoplasmic proportion. The nucleus occupies basal 1/3rd of the cell with reversed nuclear polarity but nuclear palisading is not a constant feature (**Fig-13** and **Fig-15**). The cells are bordered by differentiated ectomesenchymal cells (Preodontoblasts) producing dentin matrix on one side and prominent stratum intermedium on the other side. The basement membrane appears disintegrated.	**Secretory ameloblasts (Late bell stage):** The zone of secretory ameloblast cells extends from the transition point of presecretory ameloblasts on either side. These cells are columnar shaped cells containing round nucleus and conical cytoplasmic projections (Tomes' process). The nucleus occupies basal 1/3rd of the cell with reversed nuclear polarity and palisading (**Fig-14**). (However, palisading is not a typical feature of initial secretory ameloblasts with Tomes' process). (**Fig-16**) The cytoplasm of the cell appear granular. The cells are bordered by enamel matrix on one side and a thin layer of stratum intermedium on the other side.
 (**Fig-11:** The photomicrograph showing presecretory ameloblasts (100x H&E).	 (**Fig-12:** The photomicrograph showing secretory ameloblasts (100x H&E).

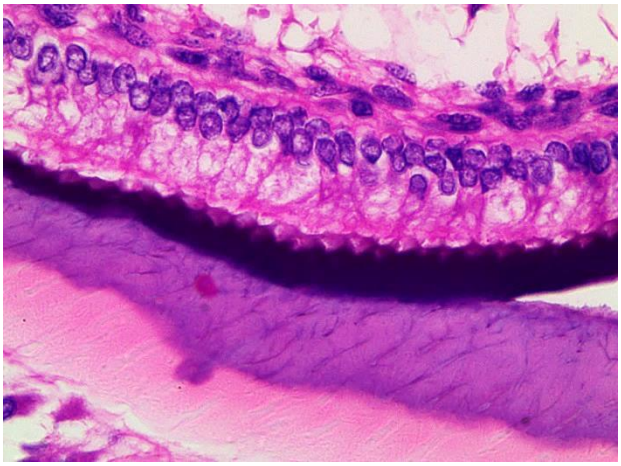


(**Fig-13**: The photomicrograph showing presecretory ameloblasts with apparent pseudostratification (400x H&E).

(**Fig-14:** The photomicrograph showing secretory ameloblasts showing palisading (400x H&E).



(**Fig-15:** The photomicrograph showing presecretory ameloblasts (400x H&E).



(**Fig-16:** The photomicrograph showing secretory ameloblasts (400x H&E).

CHART-II

Results of ameloblastoma cases with their expression pattern

CASENO	CELL DESCRIPTION	SIMILARITY TO NORMAL CELL	E-Cadherin	CK-14	CK-19
Case-1	It consists of cystic growth pattern with more tall columnar cells containing long and slender nucleus with increased cytoplasmic proportions. The nucleus show reversed polarity and apparent palisading and occupies basal 1/3rd of the cell.	Presecretory ameloblasts (PSA)	P++(M)	P++	P+
	The other outer cell is less columnar compared to previous cells. It contains oval shaped nucleus with reversed polarity and apparent palisading. The nucleus occupied the basal 1/3rd of the cell. The apical end of the cell is scalloped. The inner cells are composed of stellate reticulum-like and squamous cells. **Note:** the stellate reticulum-like and squamous cells reacted strongly to CK-14, CK-19 and EC.	Secretory ameloblasts (SA)	P++(M)	P++	P++
Case-2	It consists of follicular pattern with inner cells that were either stellate reticulum-like or round darkly stained cells associated with or without keratinizing cells. The outer cells are cuboidal or short columnar shaped with round to squared nucleus. The nucleus almost fills the entire cell. **Note:** the stellate reticulum-like or darkly stained round cells reacted negatively to CK-14, CK-19 and EC, whereas the keratinizing cells strongly reacted CK-14 and CK-19 but showed mild intense reaction to EC.	Inner enamel epithelium (IEE)	N	P++	N

Case-3	It consists of follicular and plexiform pattern with outer and inner cells. The outer cells are tall columnar cells containing oval shaped nucleus with reversed polarity and apparent palisading. The nucleus occupied the basal 1/3rd of the cell. The cytoplasm is vacuolated in some areas. The apical end of the cell is scalloped. The other outer cells are short columnar cells with round to oval shaped nucleus which occupies almost the entire cell. The inner cells are stellate reticulum-like cells.	Secretory ameloblasts (SA) Inner enamel epithelium (IEE)	P+(M) P+ (C)	P++ P++	N=P N
Case-4	It consists of plexiform pattern with outer and inner cells. The outer cells are tall columnar cells containing long and slender nucleus. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell. The apical end of the cell is scalloped. The other outer cells are short columnar cells with round to oval shaped nucleus which occupies almost the entire cell. The inner cells are stellate reticulum-like cells.	Preameloblasts as in early bell stage (PA-EBS) Inner enamel epithelium (IEE)	N N	P++ P++	P++ N
Case-5	It consists of plexiform pattern with outer and inner cells. The outer cell layer without definable cytoplasm containing round to oval washed-out staining nucleus with multilayered arrangement. The inner cells are stellate reticulum-like cells.	Unclassifiable	N	P++	P++
Case-6	It consists of a cystic growth pattern with inner stellate reticulum-like cells and outer tall columnar cells containing long and slender nucleus. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell.	Preameloblasts as in early bell stage (PA-EBS)	P+(C)	P ++	P++

	The other cells are short columnar cells with round to oval shaped nucleus which occupies almost the entire cell.	Inner enamel epithelium	P++(C)	P++	P++
Case-7	It consists of follicular and plexiform pattern with outer and inner cells. The outer cells are tall columnar cells containing oval shaped nucleus with reversed polarity and apparent palisading. The cytoplasm is vacuolated. The apical end of the cell is scalloped. In other fields the nuclei appeared staggered and the cells were slightly shorter. The other cell types are characterized by either short columnar or darkly stained cells with round nucleus and round cell outline especially the inner cells.	Secretory ameloblasts (vacuolated) (SA) Preameloblasts (PA-LBS) Inner enamel epithelium (IEE) Unclassifiable	N N N N	N P+ P++ P++	N N P+ P+
Case-8	It consists of plexiform pattern with outer cells are tall columnar cells containing long and slender nucleus. The nucleus show reversed polarity but appeared pseudostratified and occupies almost half of the cell. Some areas show multilayered peripheral cells. The other outer cells are round nucleated cells without definable cytoplasm. The third type of outer cells is tall columnar cells containing round to oval nucleus. The nucleus show reversed polarity with apparent palisading and occupies basal 1/3rd of the cell. The inner cells are composed of stellate reticulum-like and round nucleated cells without definable cytoplasm.	Unclassifiable Unclassifiable Secretory ameloblasts (SA)	P+(C) P+(M) N	P++ P++ P++	

Case-9	It consists of follicular pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell. The inner cells are stellate reticulum-like, squamous cells and round cells.	Preameloblasts (PA-LBS)	N=P	P++
Case-10	It consists of cystic growth pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell.	Preameloblasts (PA-LBS)	P+(C)	N
Case-11	It consists of cystic growth pattern with tall columnar cells containing long and slender nucleus. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell. The inner cells are stellate reticulum-like and squamous cells.	Preameloblasts as in early bell stage (PA-EBS)	P+(C)	P++
Case-12	It consists of cystic growth pattern with outer cells are cuboidal or short columnar shaped with round to squared nucleus. The nucleus almost fills the entire cell. The inner cells are stellate reticulum-like and keratinizing cells.	Inner enamel epithelium (IEE)	P++(C)	P++
Case-13	It consists of follicular and plexiform pattern with outer cells are cuboidal or short columnar shaped with round to squared nucleus. The nucleus almost fills the entire cell. The inner cells are stellate reticulum-like and acanthomatous cells. The other cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell.	Inner enamel epithelium (IEE) Preameloblasts (PA-LBS)	P+ (C) P+(C)	P++ P++

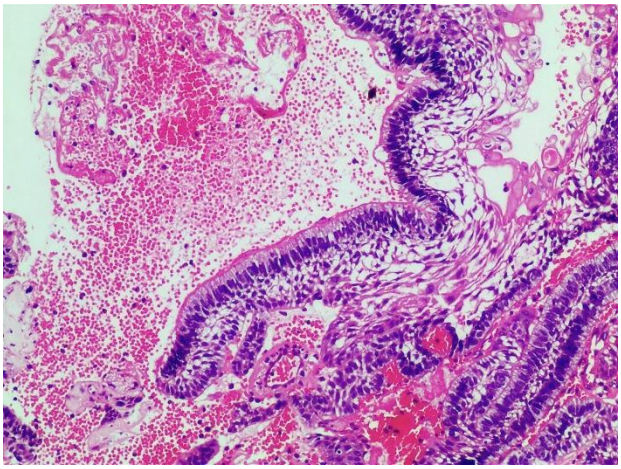
Case-14	It consists of cystic growth pattern with outer cells are more tall columnar cells containing long and slender nucleus with increased cytoplasmic proportions. The nucleus show reversed polarity and apparent palisading and occupies basal 1/3rd of the cell. The inner cells are stellate reticulum-like cells.	Presecretory ameloblasts (PSA)	P++(M)	P++	
Case-15	It consists of cystic growth pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell.	Preameloblasts (PA-LBS)	P++(C)	P++	
Case-16	It consists of cystic growth pattern with outer cells of the ameloblastoma are cuboidal cells with centrally placed nucleus. The nucleus almost fills the cell. The inner cells are stellate reticulum-like and keratinizing cells.	Inner enamel epithelium (IEE)	P+(C)	P++	
Case-17	It consists of plexiform pattern with outer cells are more tall columnar cells containing long and slender nucleus with increased cytoplasmic proportions. The nucleus show reversed polarity and apparent palisading and occupies basal 1/3rd of the cell. The inner cells are stellate reticulum-like and round cells.	Presecretory ameloblasts (PSA)	P++(C)	P++	P++
Case-18	It consists of follicular, plexiform and basaloid pattern with outer cells cuboidal or short columnar shaped with round to squared nucleus. The nucleus almost fills the entire cell. The other outer cells are round nucleated cells without definable cytoplasm. The inner cells are stellate reticulum-like and round cells.	Inner enamel epithelium (IEE) Unclassifiable	P+(C) P+(C)	P++ P++	N N

Case-19	It consists of follicular pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell. The inner cells are stellate reticulum-like cells.	Preameloblasts (PA-LBS)	N	P+	N
Case-20	It consists of follicular pattern with outer cells cuboidal or short columnar shaped with round to oval nucleus. The nucleus almost fills the entire cell. The inner cells are stellate reticulum-like and acanthomatous cells.	Inner enamel epithelium (IEE)	P+(C)	P++	P+

The photographic illustration is made showing different expression pattern of all the cases of ameloblastomas- Refer-**CHART-III.**

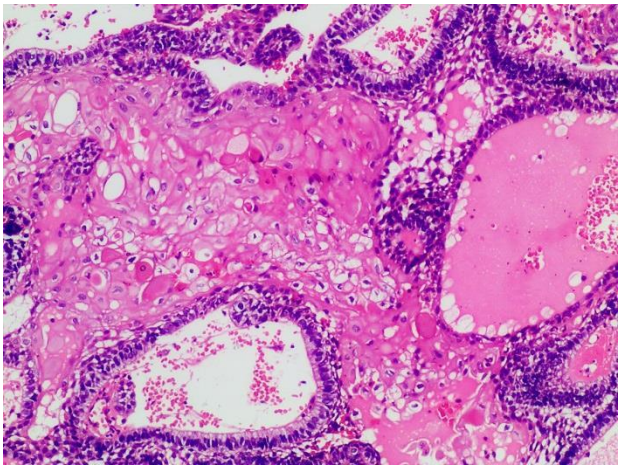
CASE-1 **CHART-III**

It consists of cystic growth pattern with more tall columnar outer cells containing long and slender nucleus with increased cytoplasmic proportions. The nucleus show reversed polarity and apparent palisading and occupies basal 1/3rd of the cell (**Fig-19**). The inner cells are composed of stellate reticulum-like and squamous cells. The normal equivalent of the outer cells resembles **"Presecretory ameloblasts (PSA) of tooth germ."**

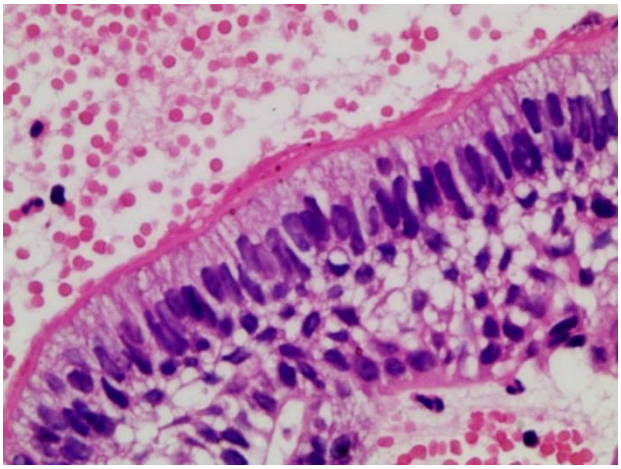


(**Fig-17**: The photomicrograph shows ameloblastoma island with presecretory-like ameloblasts and stellate reticulum-like cells (x100, H&E).

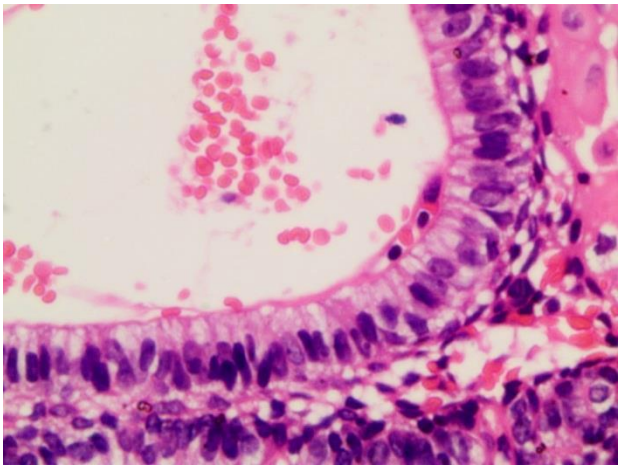
The other outer cell is less columnar compared to the previous cell described. It contains oval shaped nucleus with reversed polarity and apparent palisading. The nucleus occupies the basal 1/3rd of the cell (**Fig-20**). The apical end of the cell is scalloped. The inner cells are composed of stellate reticulum-like and squamous cells. The normal equivalent of the outer cells resembles **"Secretory ameloblasts (SA) of tooth germ."**



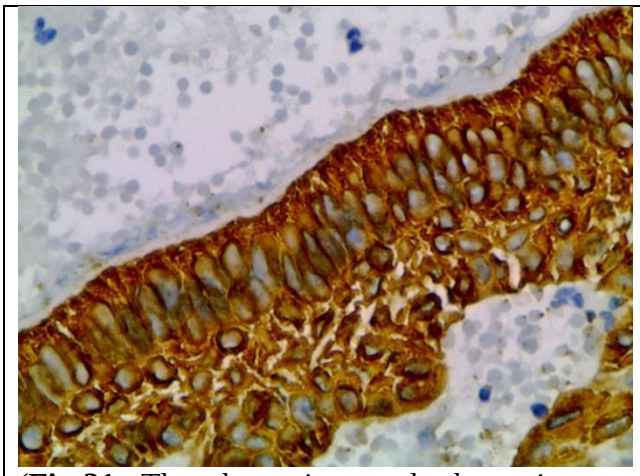
(**Fig-18:** The photomicrograph shows ameloblastoma island with secretory-like ameloblasts, stellate reticulum-like and acanthomatous cells (x100, H&E).



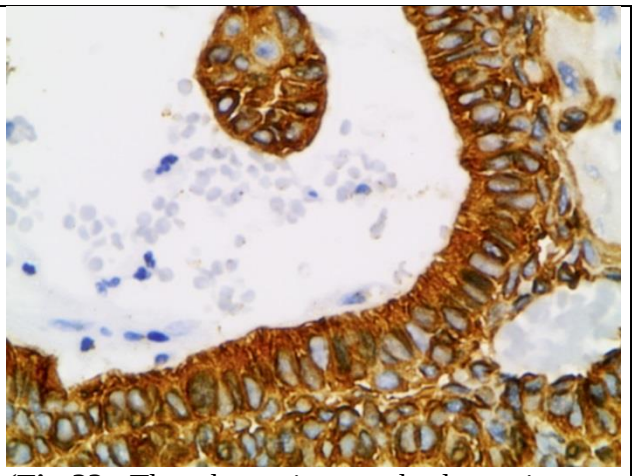
(**Fig-19:** The photomicrograph shows outer cells of ameloblastoma island resembling presecretory ameloblasts (x400, H&E).



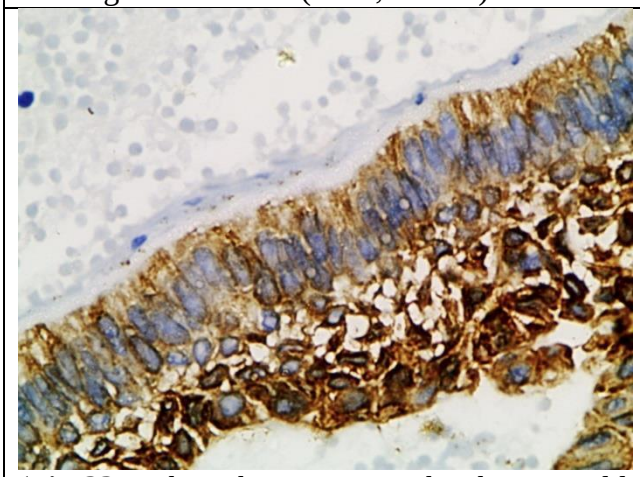
(**Fig-20:** The photomicrograph shows outer cells of ameloblastoma island resembling secretory ameloblasts(x400, H&E).



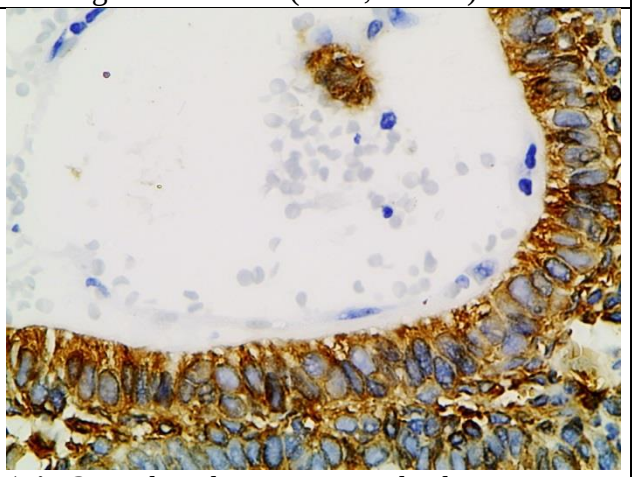
(**Fig-21:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



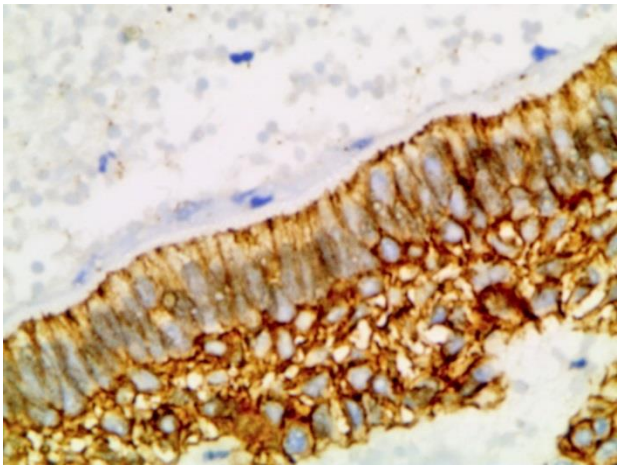
(**Fig-22:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



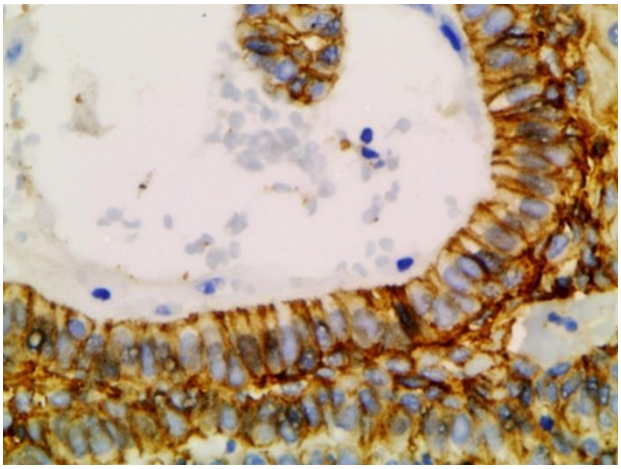
(**Fig-23:** The photomicrograph shows mild staining in outer cells but intense in SR-like cells (x400, CK-19).



(**Fig-24:** The photomicrograph shows intense staining in outer cells (x400, CK-19).



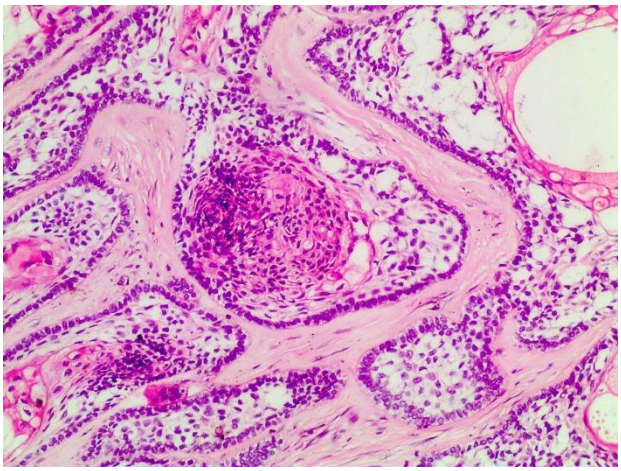
(**Fig-25:** The photomicrograph shows intense staining in cytoplasmic region of outer cells (x400, E-Cadherin).



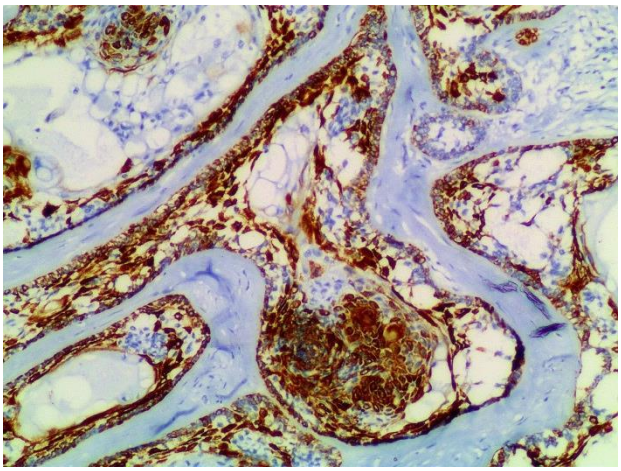
(**Fig-26:** The photomicrograph shows intense staining (x400, E-Cadherin). (Note:the stainig is more intense than the Fig-25).

CASE-2

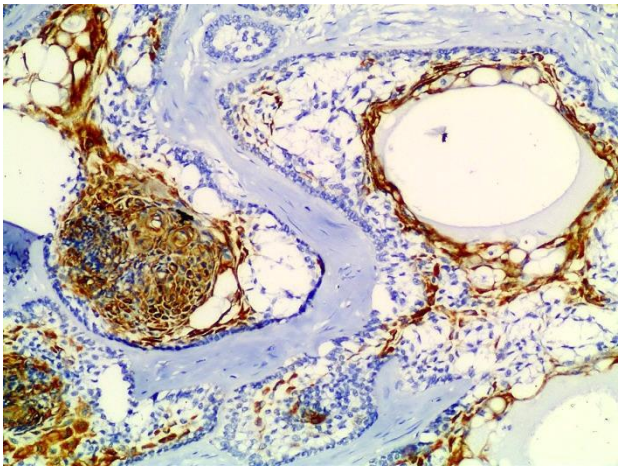
It consists of follicular pattern with inner cells that were either stellate reticulum-like or round darkly stained cells associated with or without keratinizing cells. The outer cells are cuboidal or short columnar shaped with round to squared nucleus. The nucleus almost fills the entire cell (**Fig-27** and **Fig-31**). The normal equivalent of the outer cell resembles **"Inner enamel epithelium of tooth germ (IEE)"**



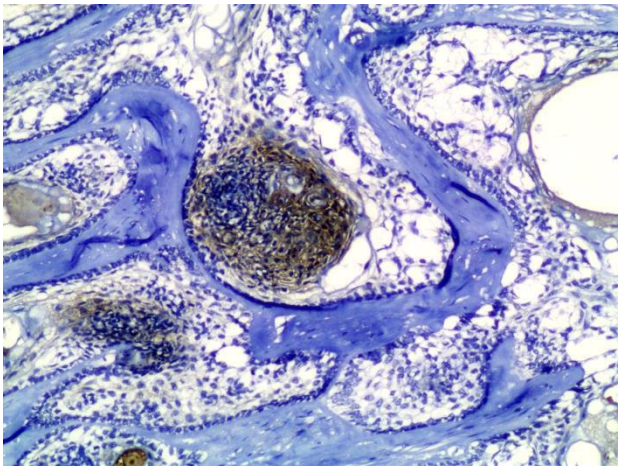
(**Fig-27:** The photomicrograph shows ameloblastoma island with inner enamel epithelium-like ameloblasts, stellate reticulum-like and acanthomatous cells (x100, H&E).



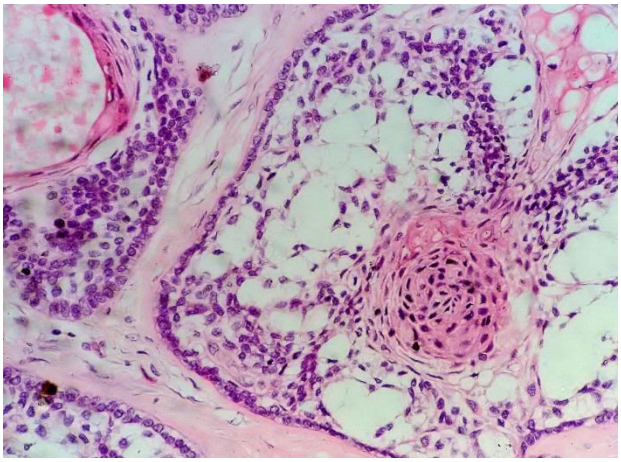
(**Fig-28:** The photomicrograph shows ameloblastoma island with intense staining of outer cells (x100, CK-14).



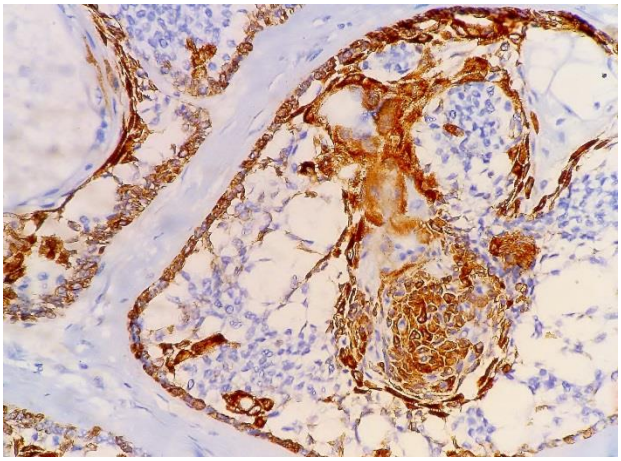
(**Fig-29:** The photomicrograph shows ameloblastoma island with negative staining of outer cells (x100, CK-19).



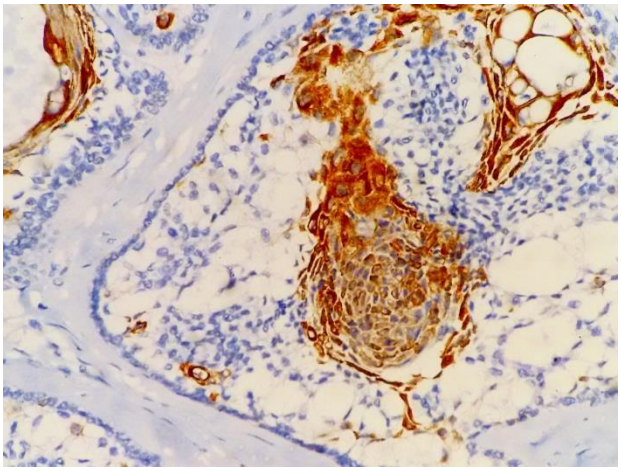
(**Fig-30:** The photomicrograph shows ameloblastoma island with negative staining of outer cells (x100, E-Cadherin).



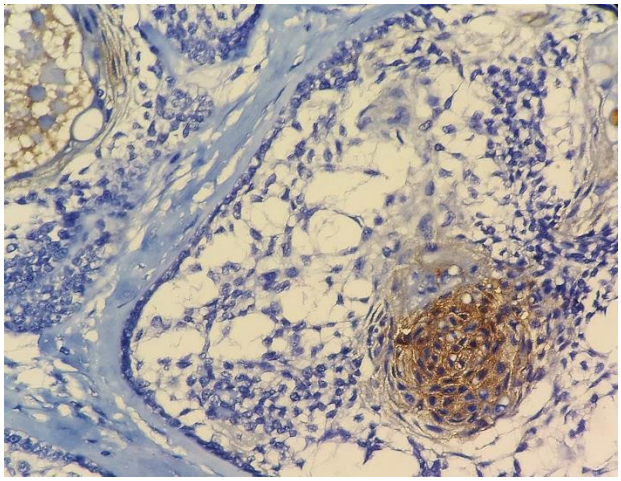
(**Fig-31:** The photomicrograph shows ameloblastoma island with inner enamel epithelium-like ameloblasts, stellate reticulum-like and acanthomatous cells (x400, H&E).



(**Fig-32:** The photomicrograph shows intense staining in outer cells and acanthomatous cells (x400, CK-14).



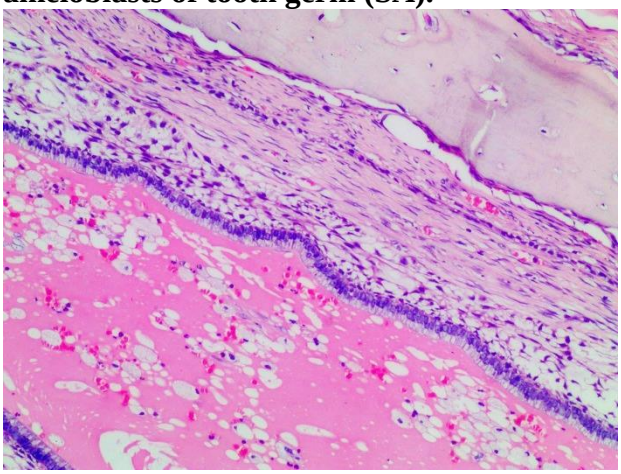
(**Fig-33:** The photomicrograph shows intense staining in acanthomatous cells (x400, CK-19).



(**Fig-34:** The photomicrograph shows mild staining in acanthomatous cells (x400, E-Cadherin).

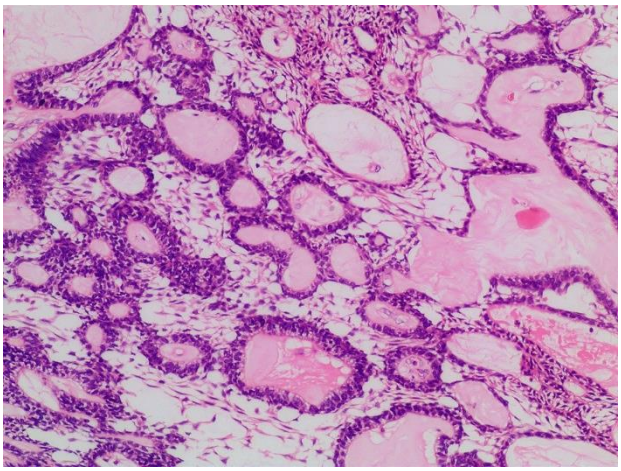
CASE-3

It consists of follicular and plexiform pattern with outer and inner cells. The outer cells are tall columnar cells containing oval shaped nucleus with reversed polarity and apparent palisading. The nucleus occupies the basal 1/3rd of the cell. The apical end of the cell is scalloped (**Fig-37**). The inner cells are stellate reticulum-like cells. The normal equivalent of the outer cells resembles **"Secretory ameloblasts of tooth germ (SA)."**

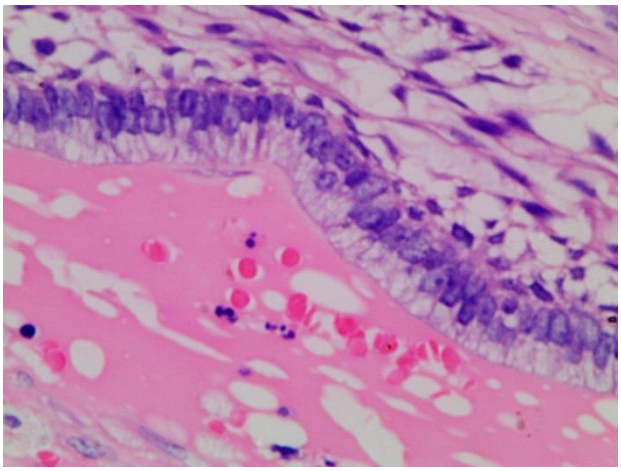


(**Fig-35:** The photomicrograph shows ameloblastoma with peripheral cell resembles secretory ameloblasts and stellate reticulum-like cells (x100, H&E).

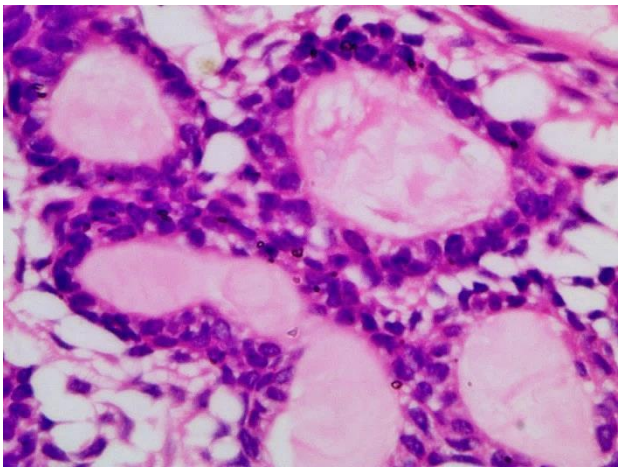
The other outer cells are short columnar cells with round to oval shaped nucleus which occupies almost the entire cell (**Fig-38**). The inner cells are stellate reticulum-like cells. The normal equivalent of the outer cells resembles **"Inner enamel epithelium of tooth germ (IEE)."**



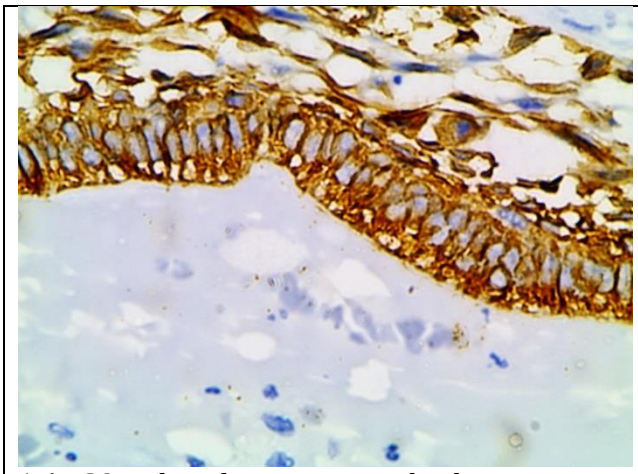
(**Fig-36:** The photomicrograph shows ameloblastoma with peripheral cell resembles inner enamel epithelium and stellate reticulum-like cells (x100, H&E).



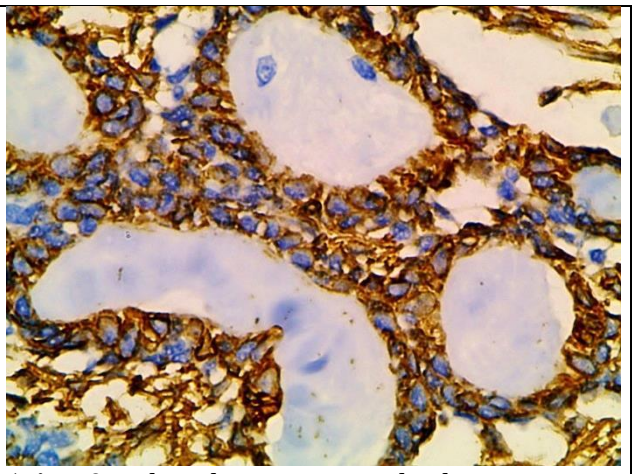
(**Fig-37:** The photomicrograph shows peripheral cells of ameloblastoma resembling secretory ameloblasts (x400, H&E).



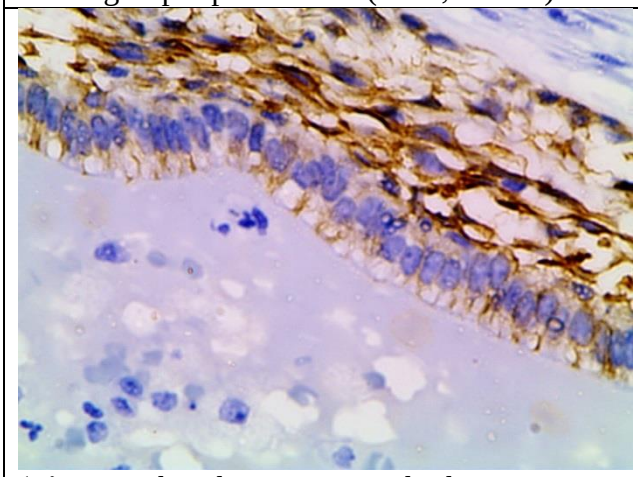
(**Fig-38:** The photomicrograph shows peripheral cells of ameloblastoma resembling inner enamel epithelium (x400, H&E).



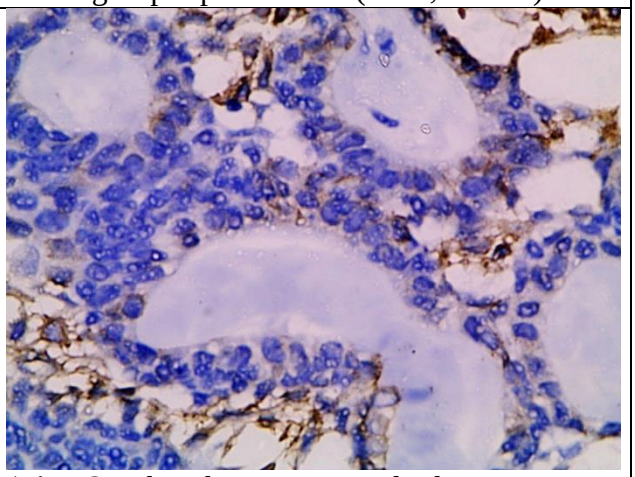
(**Fig-39**: The photomicrograph shows intense staining of peripheral cells (x400, CK-14).



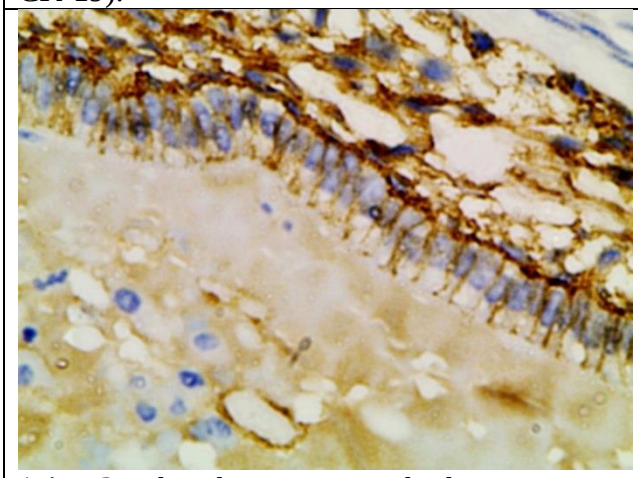
(**Fig-40:** The photomicrograph shows intense staining of peripheral cells (x400, CK-14).



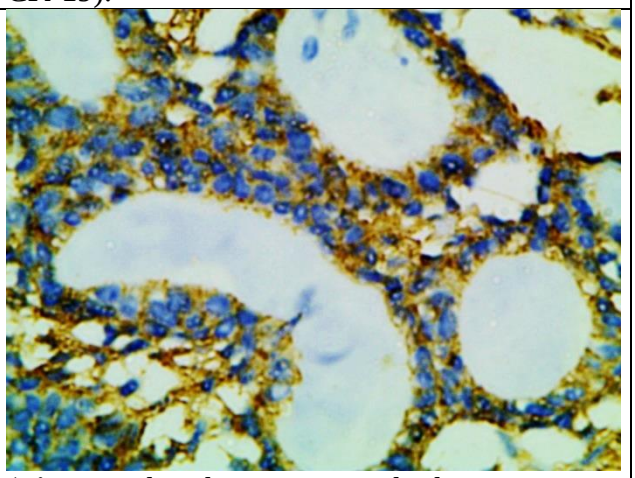
(**Fig-41:** The photomicrograph shows staining only in stellate reticulum-like cells but not in the peripheral cells of ameloblastoma (x400, CK-19).



(**Fig-42:** The photomicrograph shows staining only in stellate reticulum-like cells but not in the peripheral cells of ameloblastoma(x400, CK-19).



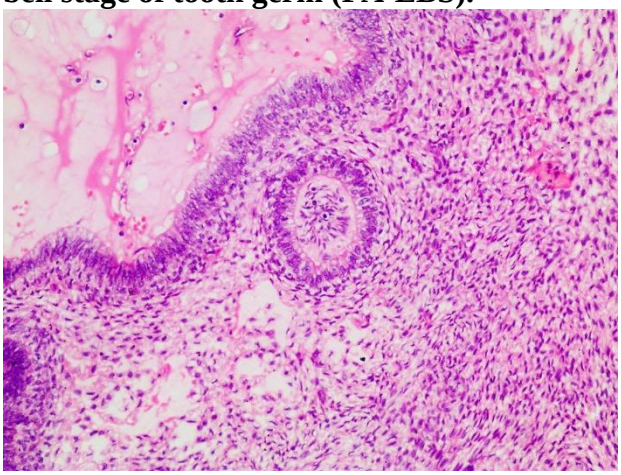
(**Fig-43:** The photomicrograph shows staining in the membraneous areas of peripheral cells (x400, E-Cadherin).



(**Fig-44:** The photomicrograph shows staining in cytoplasmic areas of peripheral cells (x400, E-Cadherin).

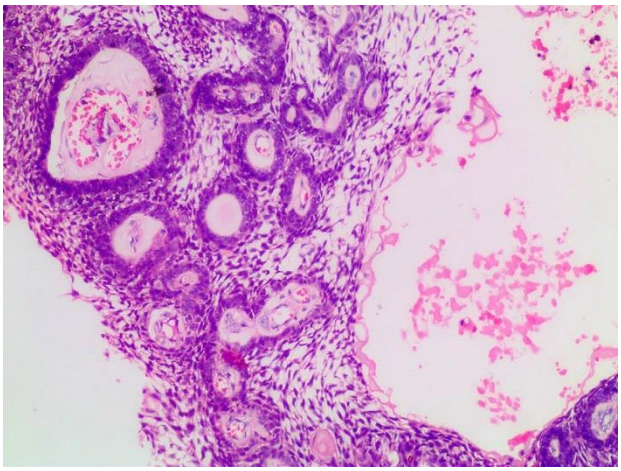
CASE-4

It consists of plexiform pattern with outer and inner cells. The outer cells are tall columnar cells containing long and slender nucleus. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell. The apical end of the cell is scalloped (**Fig-47**). The inner cells are stellate reticulum-like cells. The normal equivalent of the outer cells resembles **"Preameloblasts as in early bell stage of tooth germ (PA-EBS)."**

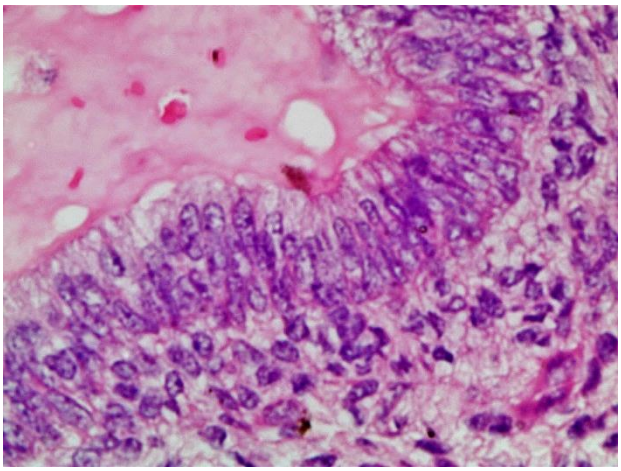


(**Fig-45:** The photomicrograph shows ameloblastoma with peripheral cell resembles preameloblasts as in early bell stage of (x100, H&E).

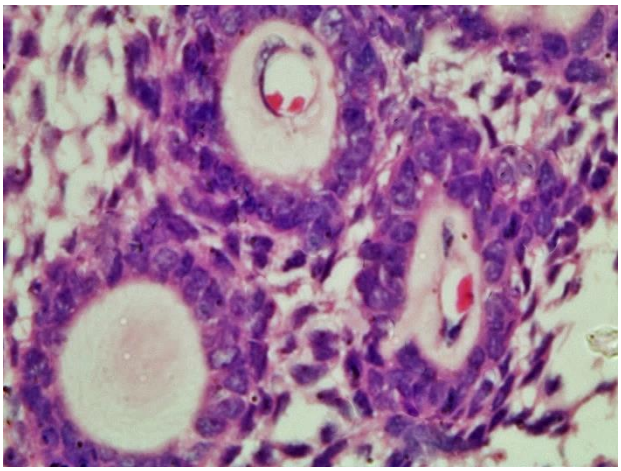
The other outer cells are short columnar cells with round to oval shaped nucleus which occupies almost the entire cell (**Fig-48**). The inner cells are stellate reticulum-like cells. The normal equivalent of the outer cells resembles **"Inner enamel epithelium of the tooth germ (IEE)."**



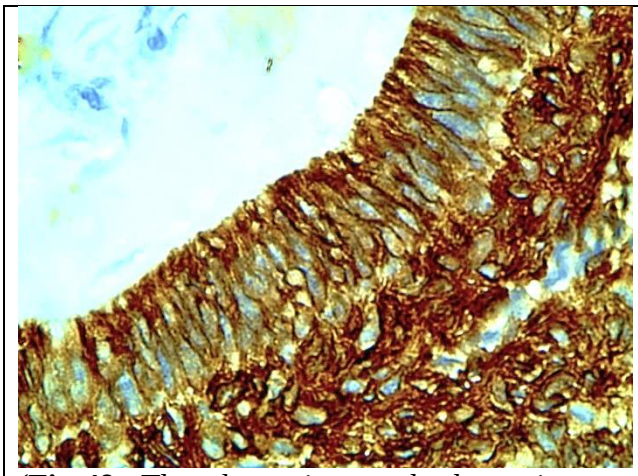
(**Fig-46:** The photomicrograph shows ameloblastoma with peripheral cell resembles inner enamel epithelium (x100, H&E).



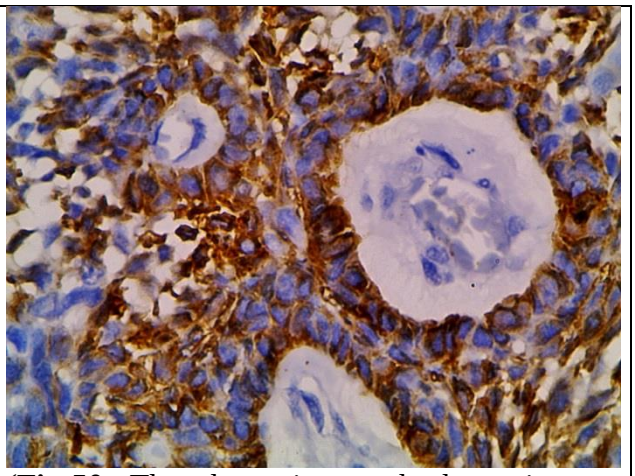
(**Fig-47:** The photomicrograph shows ameloblastoma with peripheral cell resembles preameloblasts as in early bell stage (x400, H&E).



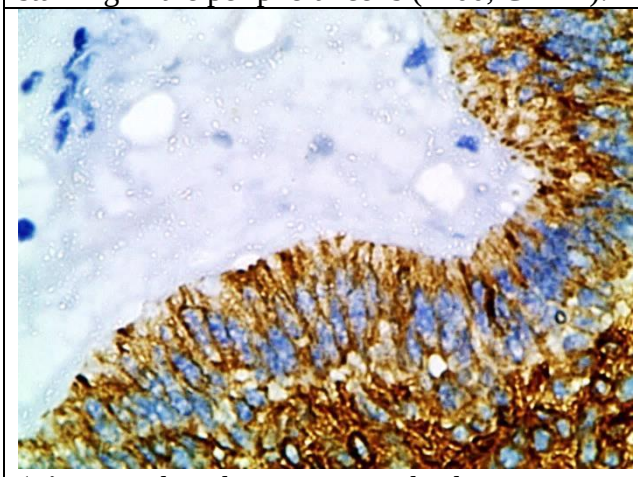
(**Fig-48:** The photomicrograph shows ameloblastoma with peripheral cell resembles inner enamel epithelium (x400, H&E).



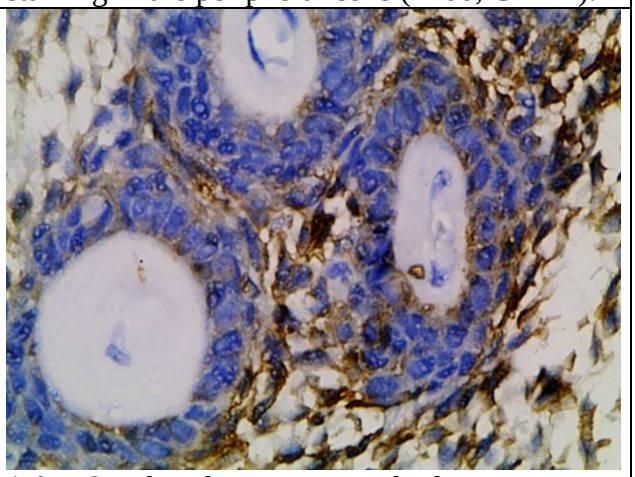
(**Fig-49:** The photomicrograph shows intense staining in the peripheral cells (x400, CK-14).



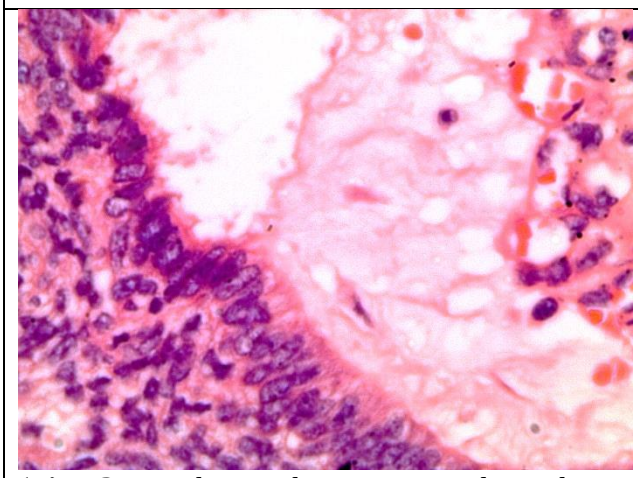
(**Fig-50:** The photomicrograph shows intense staining in the peripheral cells (x400, CK-14).



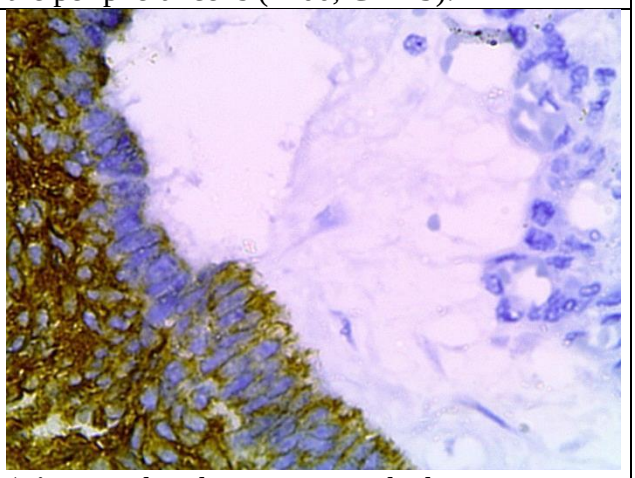
(**Fig-51:** The photomicrograph shows intense staining in the peripheral cells (x400, CK-19).



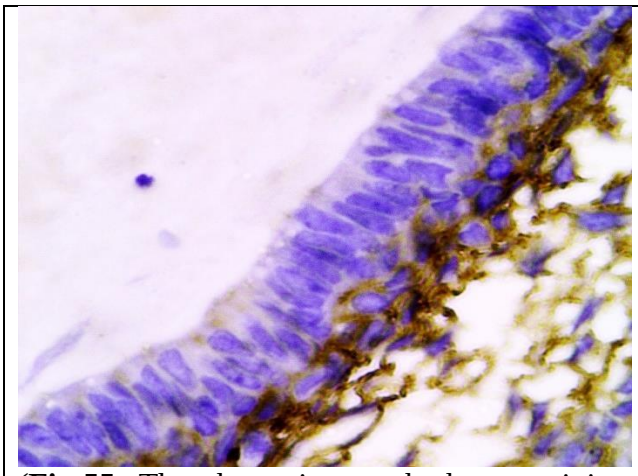
(**Fig-52:** The photomicrograph shows staining only in stellate reticulum-like cells but not in the peripheral cells (x400, CK-19).



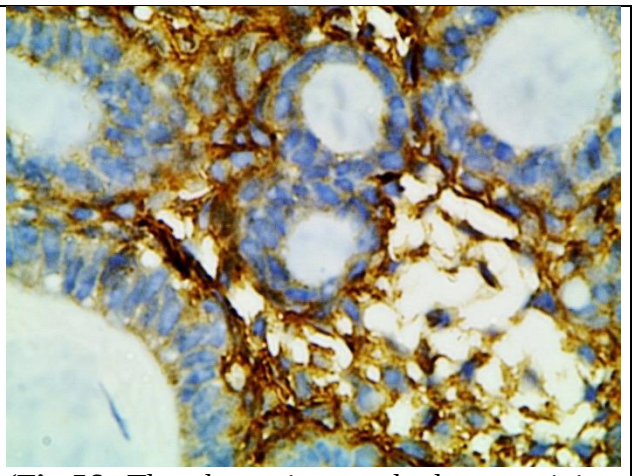
(**Fig-53:** The photomicrograph shows ameloblastoma island with peripheral cells showing various levels of differentiation (x100, CK-19).



(**Fig-54:** The photomicrograph shows variation in staining pattern of ameloblastoma with intense staining in more differentiated cells (x100, CK-19).



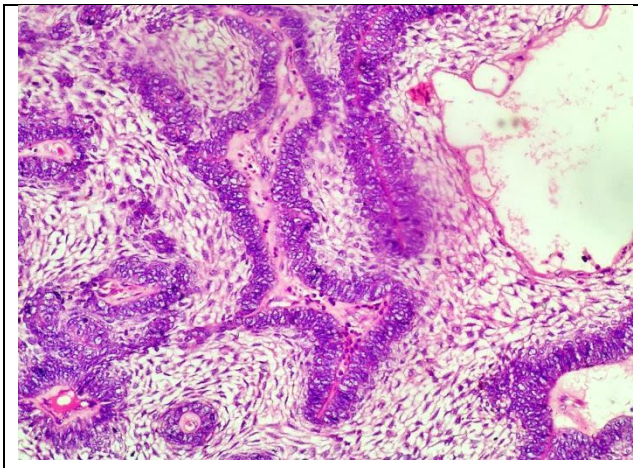
(**Fig-55:** The photomicrograph shows staining only in the stellate reticulum-like cells but not in the peripheral cells (x400, E-Cadherin).



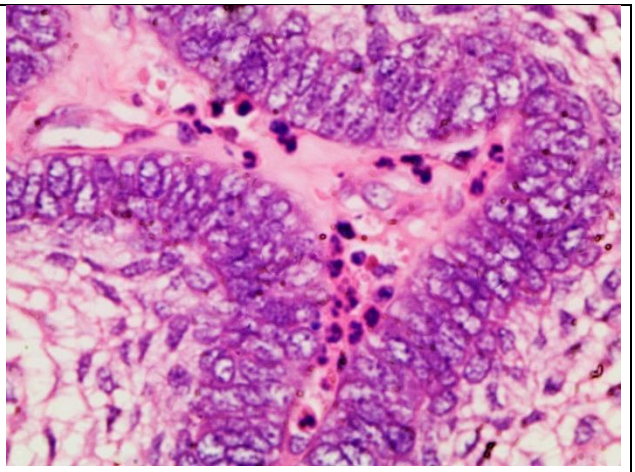
(**Fig-56:** The photomicrograph shows staining only in the stellate reticulum-like cells but not in the peripheral cells (x400, E-Cadherin).

CASE-5

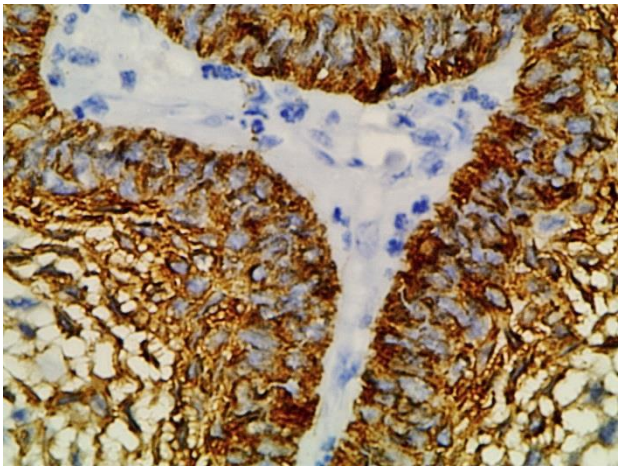
It consists of plexiform pattern with outer and inner cells. The outer cell layer is without definable cytoplasm containing round to oval washed-out staining nucleus with multilayered arrangement (**Fig-58**). The inner cells are stellate reticulum-like cells. The outer cells cannot be compared with any normal cell of developing tooth germ. **"Unclassifiable"**



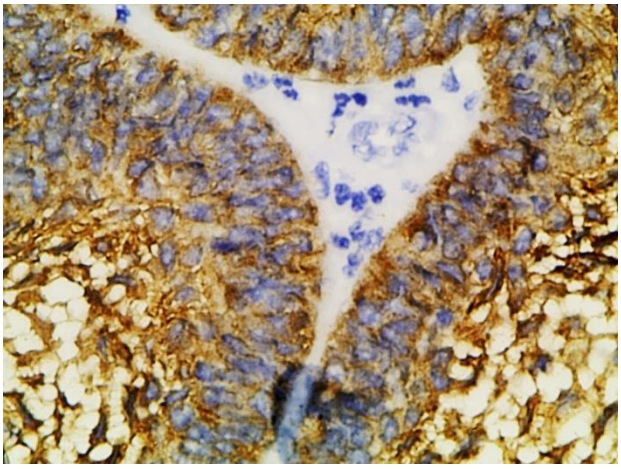
(**Fig-57:** The photomicrograph shows ameloblastoma with outer cell layer without definable cytoplasm (x100, H&E).



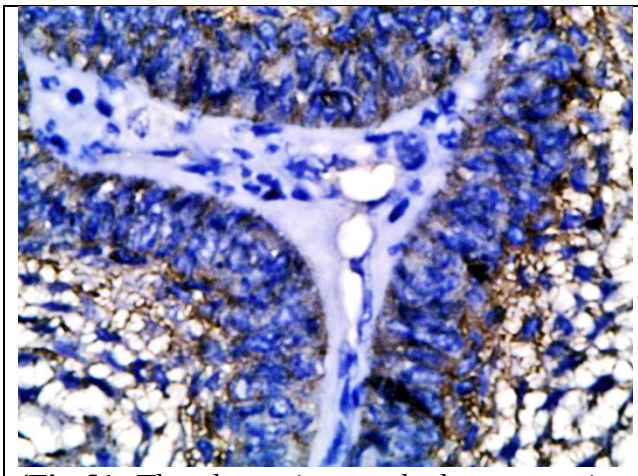
(**Fig-58:** The photomicrograph shows ameloblastoma with outer cell layer without definable cytoplasm and washed-out nucleus (x400, H&E).



(**Fig-59:** The photomicrograph shows more intense staining in the peripheral cells (x400, CK-14).



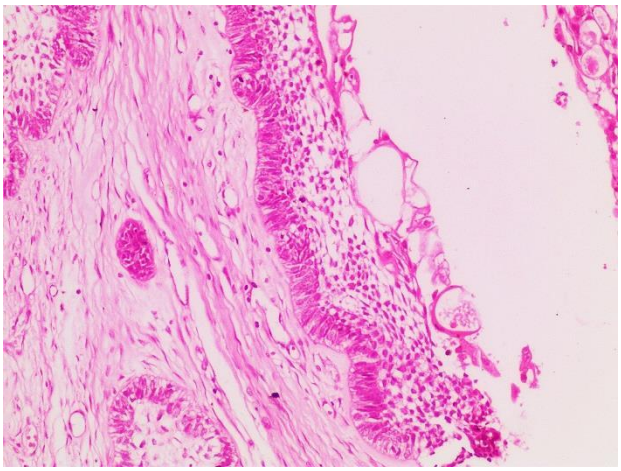
(**Fig-60:** The photomicrograph shows intense staining in peripheral cells (x400, CK-19).



(**Fig-61:** The photomicrograph shows negative staining in peripheral cells (x400, E-Cadherin).

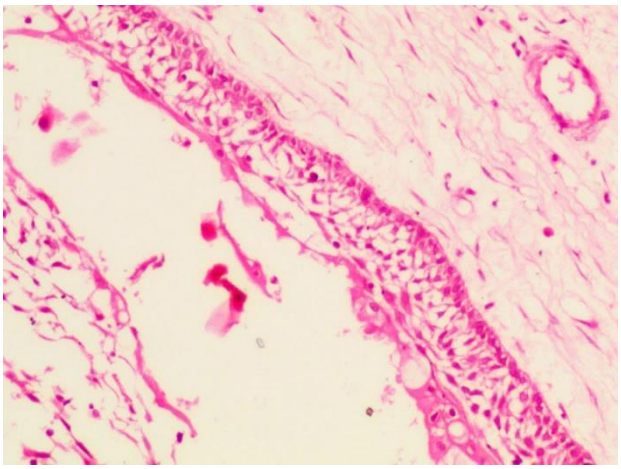
CASE-6

It consists of a cystic growth pattern with inner stellate reticulum-like cells and outer tall columnar cells containing long and slender nucleus. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell (**Fig-64**). The normal equivalent of the outer cells resembles **"Preameloblasts as in early bell stage of tooth germ (PA-EBS)."**

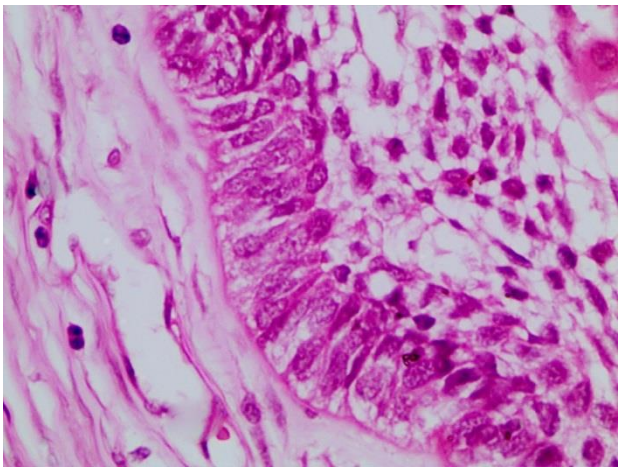


(**Fig-62:** The photomicrograph shows ameloblastoma with peripheral cell resembles preameloblasts as in early bell stage (x100, H&E).

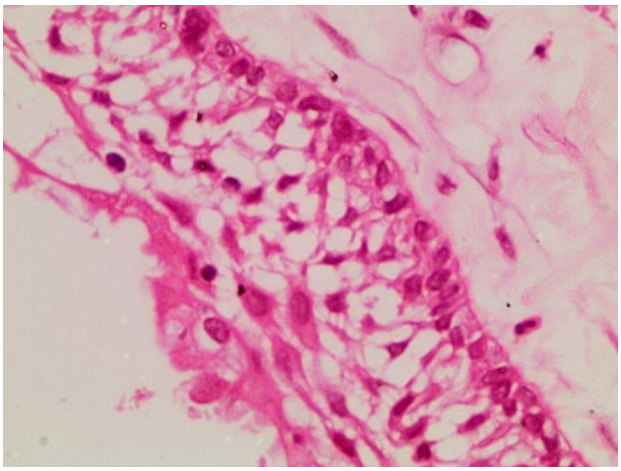
The other outer cells are short columnar cells with round to oval shaped nucleus which occupies almost the entire cell (**Fig-65**). The normal equivalent of the outer cells resembles **"Inner enamel epithelium of tooth germ (IEE)."**



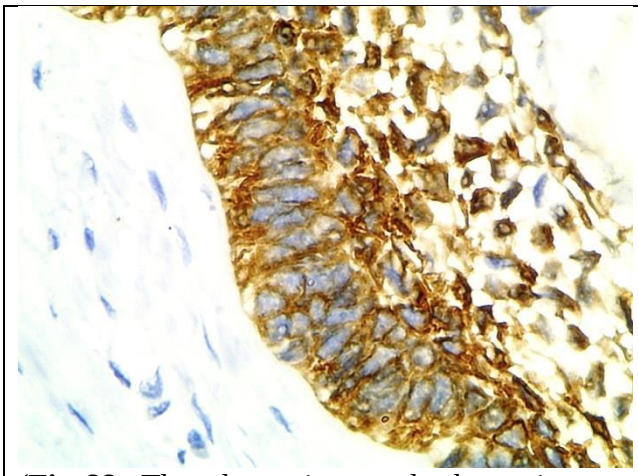
(**Fig-63:** The photomicrograph shows ameloblastoma with peripheral cell resembles inner enamel epithelium (x100, H&E).



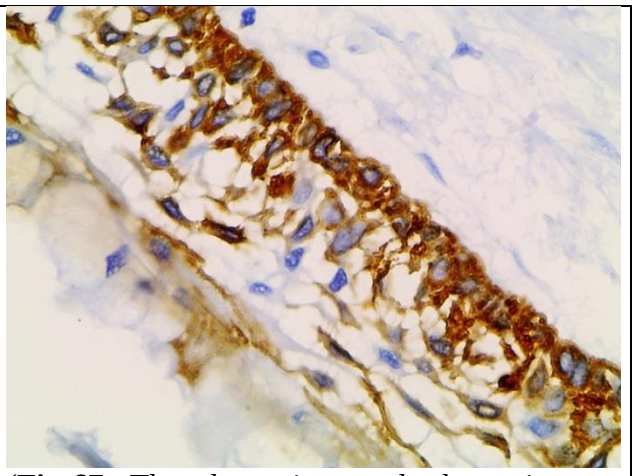
(**Fig-64:** The photomicrograph shows ameloblastoma with peripheral cell resembles preameloblasts as in early bell stage and stellate reticulum-like cells (x400, H&E).



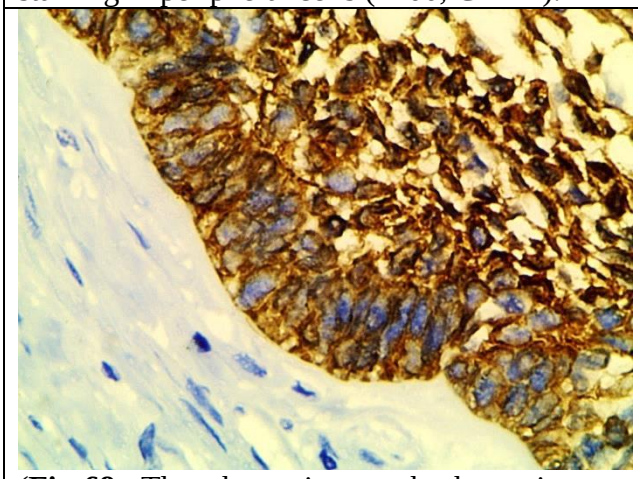
(**Fig-65:** The photomicrograph shows ameloblastoma with peripheral cell resembles inner enamel epithelium and stellate reticulum-like cells (x400, H&E).



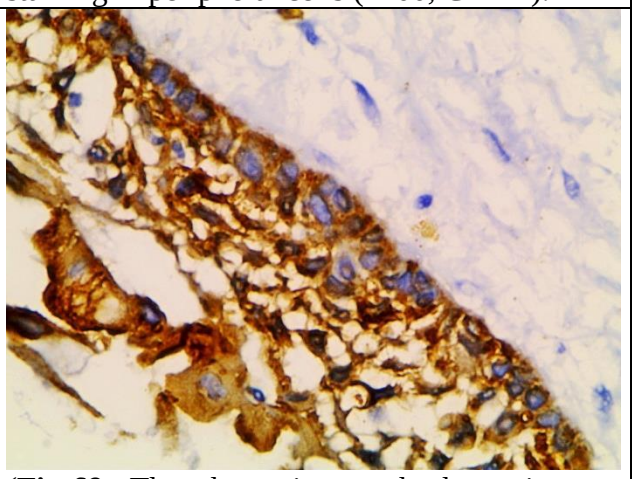
(**Fig-66:** The photomicrograph shows intense staining in peripheral cells (x400, CK-14).



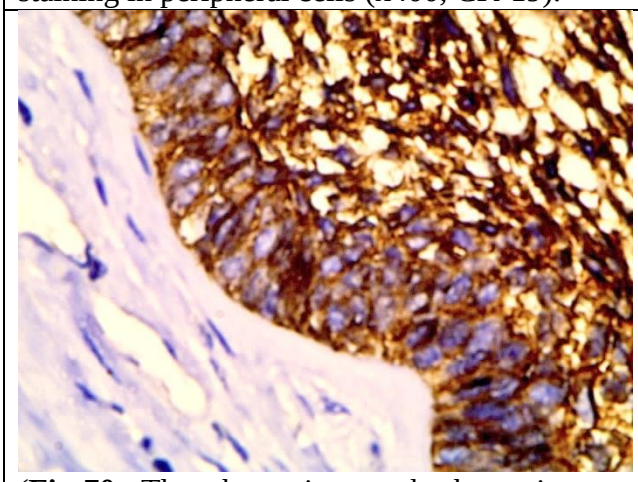
(**Fig-67:** The photomicrograph shows intense staining in peripheral cells (x400, CK-14).



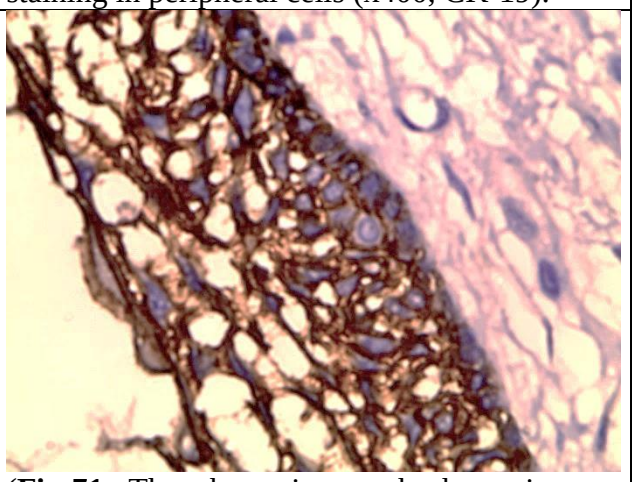
(**Fig-68:** The photomicrograph shows intense staining in peripheral cells (x400, CK-19).



(**Fig-69:** The photomicrograph shows intense staining in peripheral cells (x400, CK-19).



(**Fig-70:** The photomicrograph shows intense staining in cytoplasmic region of peripheral cells (x400, E-Cadherin).

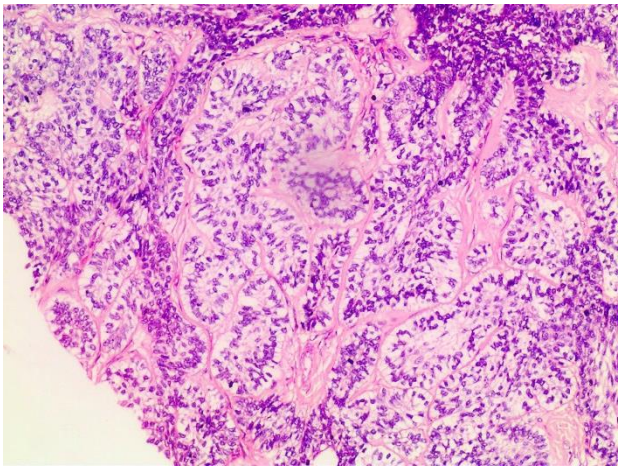


(**Fig-71**: The photomicrograph shows intense staining in cytoplasmic areas of peripheral cells (x400, E-Cadherin).

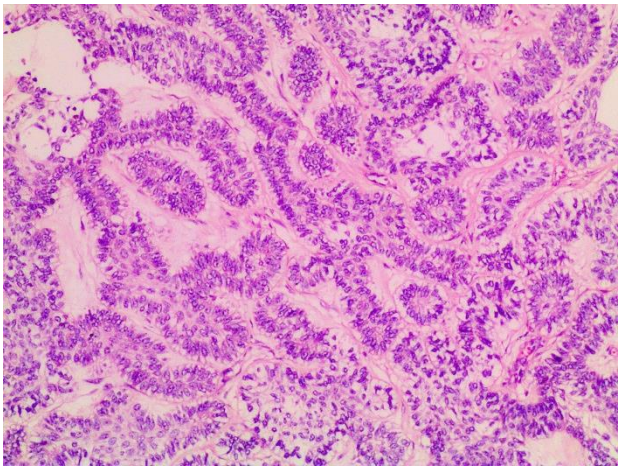
CASE-7

It consists of follicular and plexiform pattern with outer and inner cells. The outer cells are tall columnar cells containing oval shaped nucleus with reversed polarity and apparent palisading. The cytoplasm is vacuolated. The apical end of the cell is scalloped (**Fig-74**). The normal equivalent of the outer cells resembles **"Secretory ameloblasts of tooth germ (SA)."**

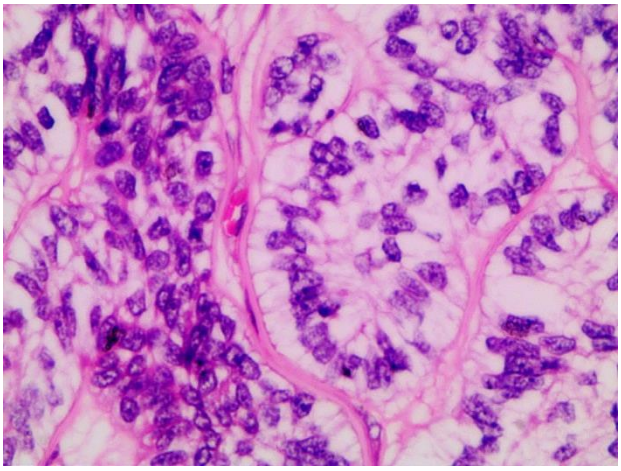
The other outer cells are columnar cells with staggered nuclei appeared shorter than the previous cells (**Fig-75**). The normal equivalent of the outer cells resembles **"Preameloblasts of tooth germ (PA-LBS)."**



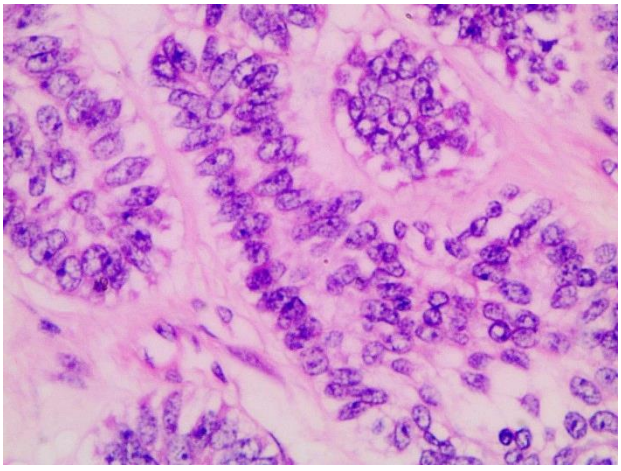
(**Fig-72:** The photomicrograph shows ameloblastoma islands with peripheral cells resembling different stages of ameloblasts of tooth germ (x100, H&E).



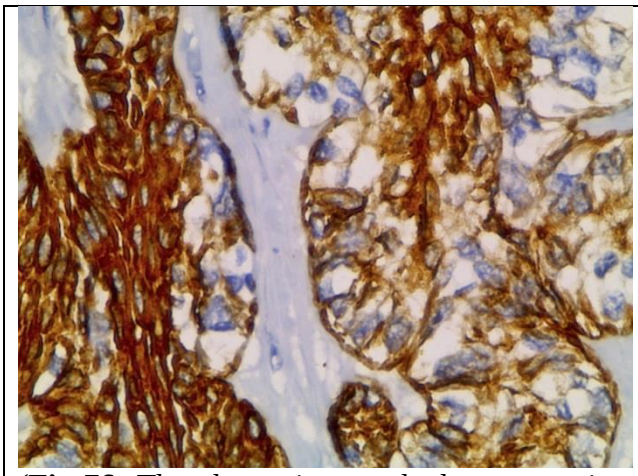
(**Fig-73:** The photomicrograph shows ameloblastoma islands with peripheral cells resembling preameloblasts (x100, H&E).



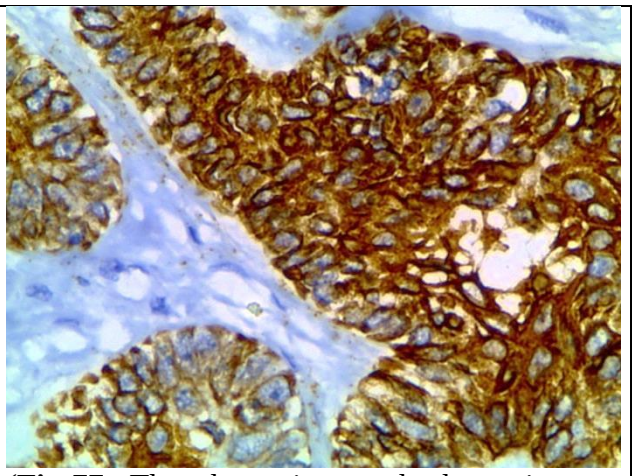
(**Fig-74:** The photomicrograph shows ameloblastoma island with peripheral cells resembling secretory ameloblasts with vacuolated cytoplasm and adjacent cells that are undifferentiated (x400, H&E).



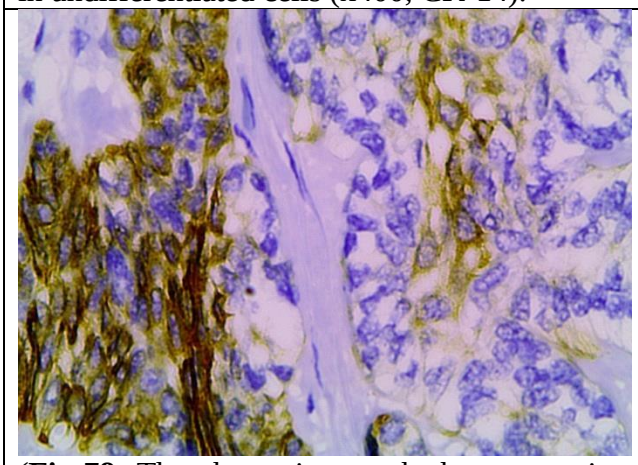
(**Fig-75:** The photomicrograph shows ameloblastoma island with peripheral cells resembling preameloblasts (x400, H&E).



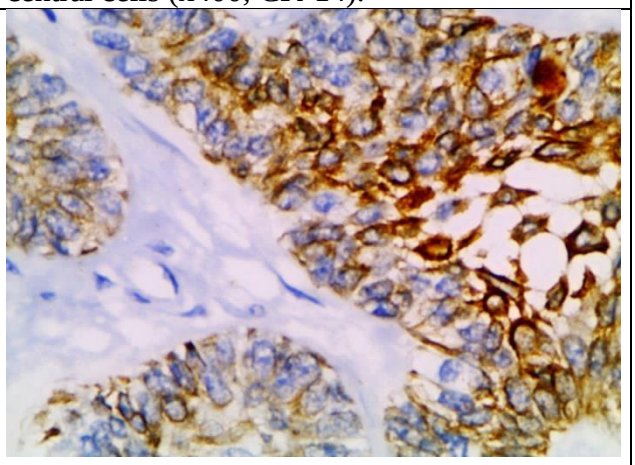
(**Fig-76:** The photomicrograph shows negative staining in vacuolated cells but intense staining in undifferentiated cells (x400, CK-14).



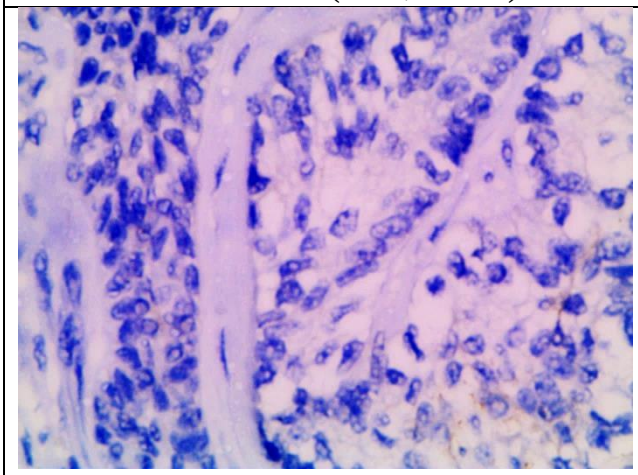
(**Fig-77:** The photomicrograph shows intense staining intense staining in peripheral and central cells (x400, CK-14).



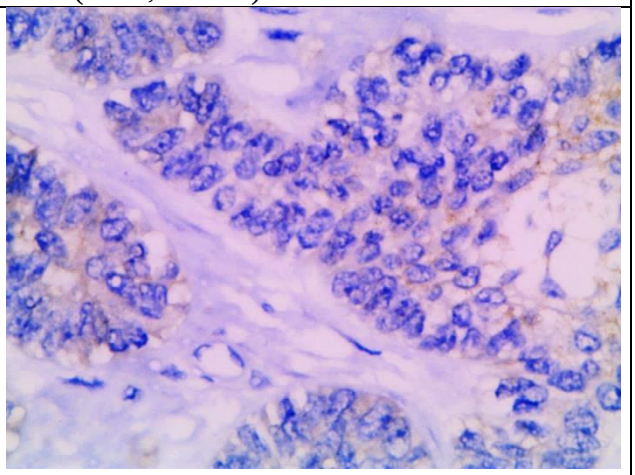
(**Fig-78:** The photomicrograph shows negative staining in vacuolated cells but intense staining in undifferentiated cells (x400, CK-19).



(**Fig-79:** The photomicrograph shows intense staining only in central stellate reticulum-like cells (x400, CK-19).

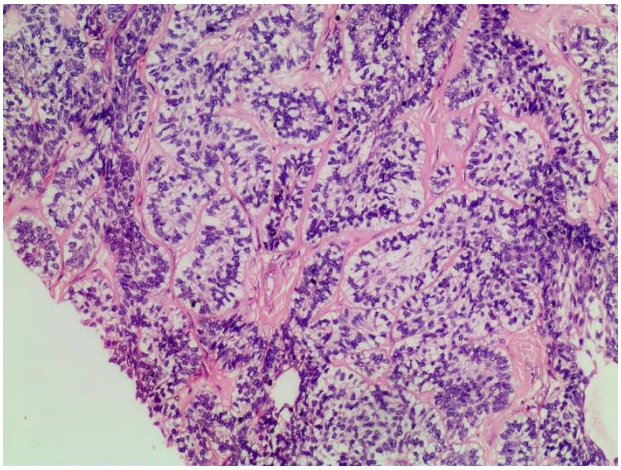


(**Fig-80:** The photomicrograph shows negative staining in both vacuolated cells and undifferentiated cells (x400, E-Cadherin).

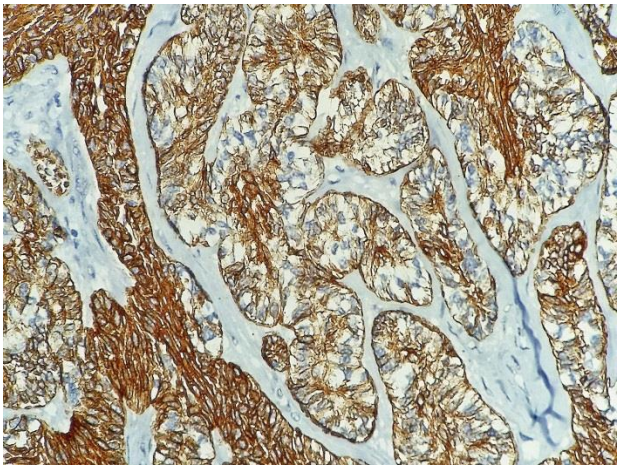


(**Fig-81:** The photomicrograph shows negative staining in both central and peripheral cells (x400, E-Cadherin).

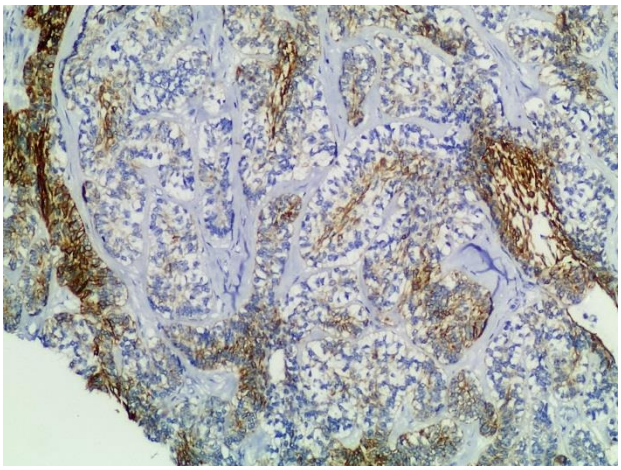
CASE-7



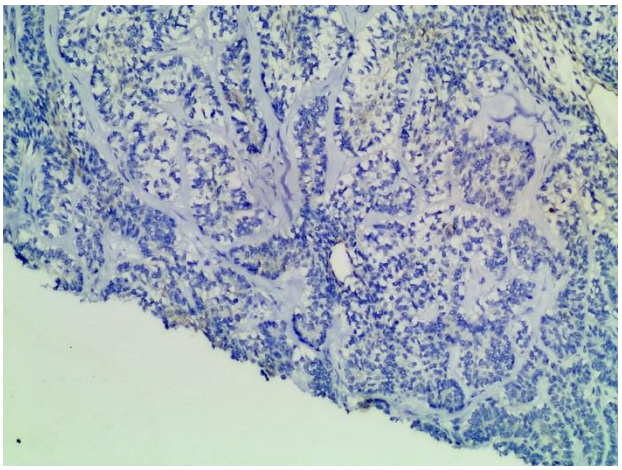
(**Fig-82:** The photomicrograph shows ameloblastoma island with peripheral cell resembling different stages of ameloblasts of tooth germ (x400, H&E).



(**Fig-83:** The photomicrograph shows negative staining of vacuolated peripheral cell and intense staining in undifferentiated cells (x400, CK-14).



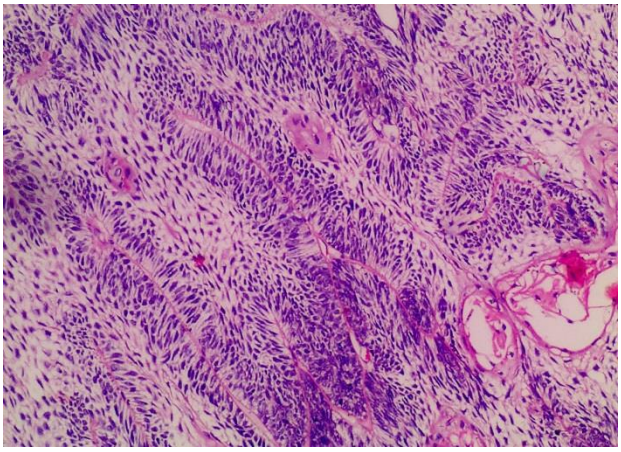
(**Fig-84:** The photomicrograph shows negative staining of vacuolated peripheral cell and less intense staining in undifferentiated cells (x400, CK19).



(**Fig-85:** The photomicrograph shows negative staining of both central and peripheral cells of ameloblastoma island (x400, E-Cadherin).

CASE-8

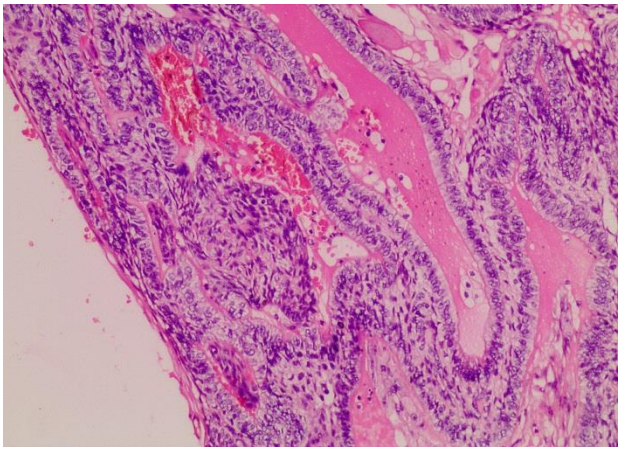
It consists of plexiform pattern with outer cells are tall columnar cells containing long and slender nucleus. The nucleus show reversed polarity but appeared pseudostratified and occupies almost half of the cell. Some areas show multilayered peripheral cells (**Fig-88**). The inner cells are composed of stellate reticulum-like and round nucleated cells without definable cytoplasm. The outer cells cannot be compared with any normal cell of developing tooth germ. **"Unclassifiable"**



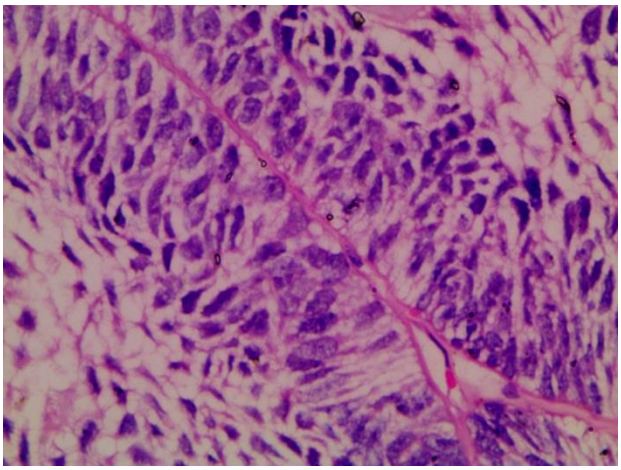
(**Fig-86:** The photomicrograph shows ameloblastoma with outer cells cannot be compared with any stage of ameloblasts (x100, H&E).

The other type of outer cells is tall columnar cells containing round to oval nucleus. The nucleus show reversed polarity with apparent palisading and occupies basal 1/3rd of the cell (**Fig-89**).

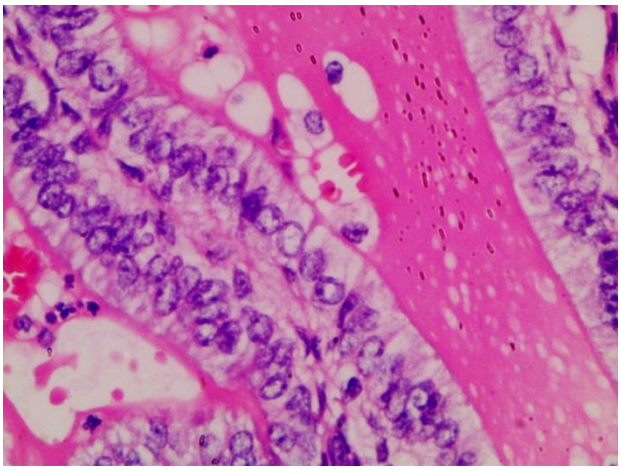
The inner cells are composed of stellate reticulum-like and round nucleated cells without definable cytoplasm. The normal equivalent of the outer cells resembles **"Secretory ameloblasts of tooth germ (SA)."**



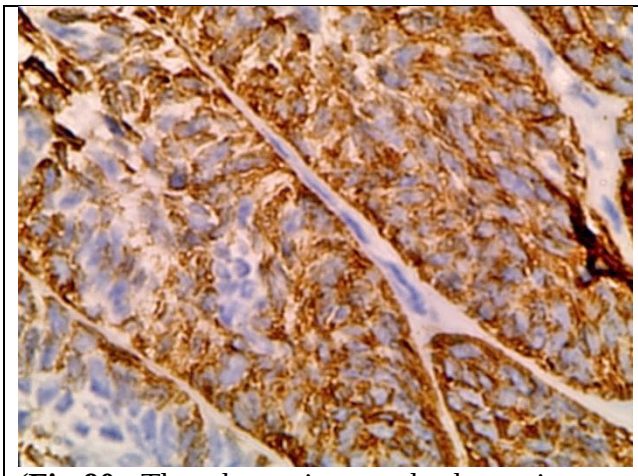
(**Fig-87:** The photomicrograph shows ameloblastoma with outer cells resembling secretory ameloblasts (x100, H&E).



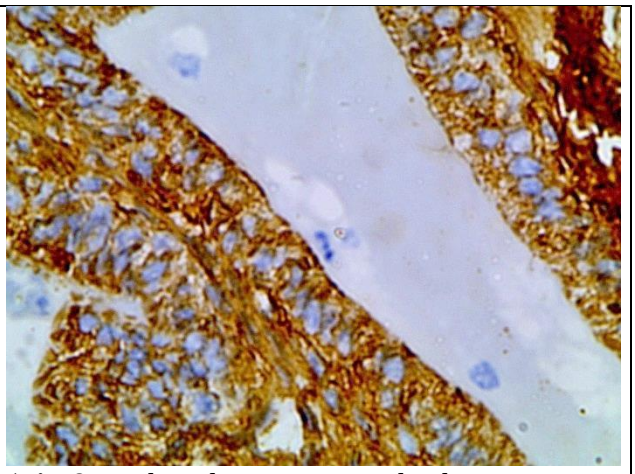
(**Fig-88:** The photomicrograph shows ameloblastoma with outer cells with multilayered arrangement (x400, H&E).



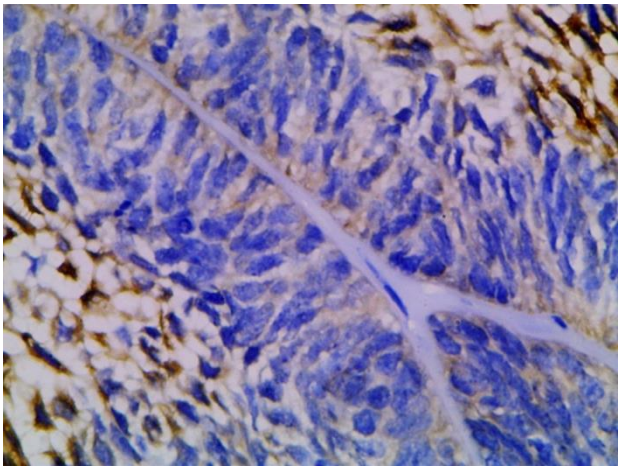
(**Fig-89:** The photomicrograph shows ameloblastoma with outer cells resembling secretory ameloblasts (x400, H&E).



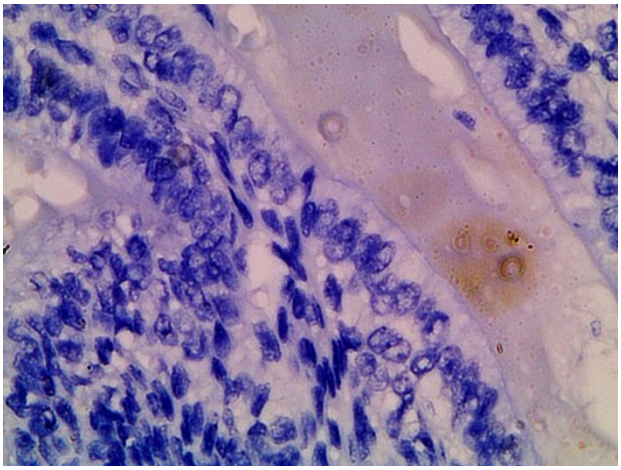
(**Fig-90:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



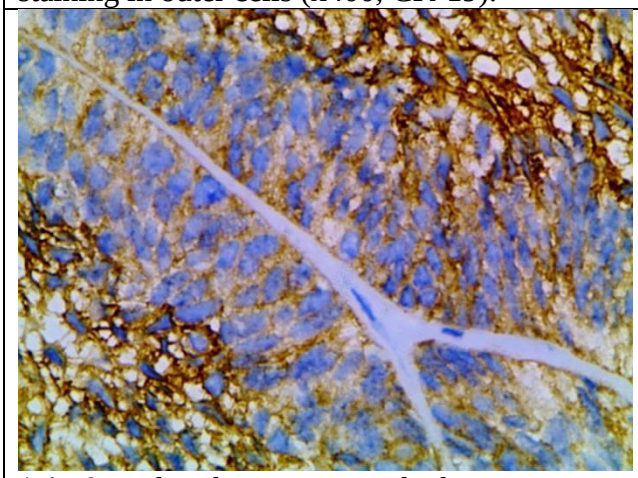
(**Fig-91**: The photomicrograph shows intense staining in both outers and inner cells (x400, CK-14).



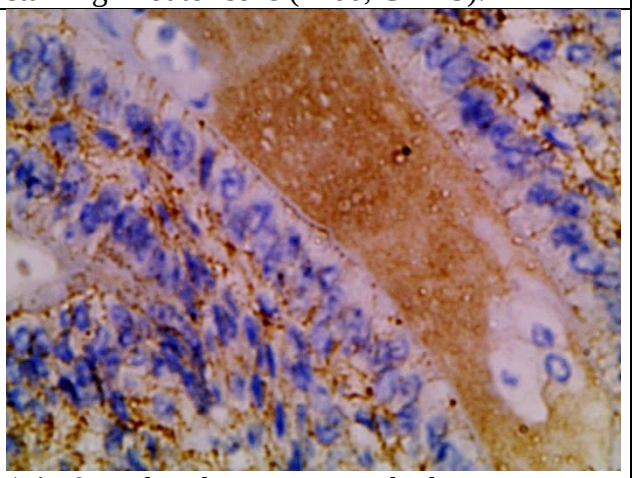
(**Fig-92:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



(**Fig-93:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



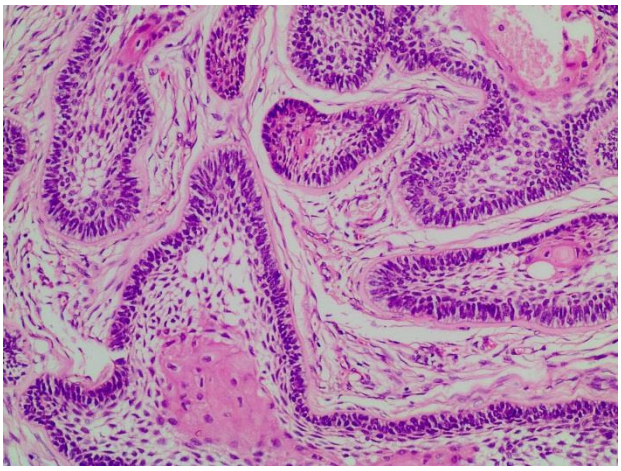
(**Fig-94:** The photomicrograph shows negative staining in outer cells but intense staining in inner cells (x400, E-Cadherin).



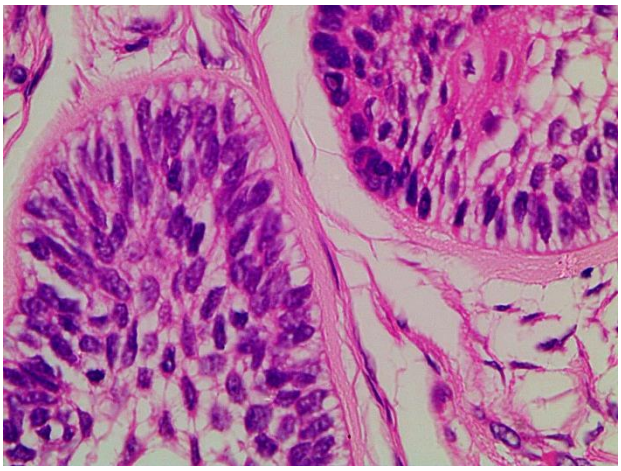
(**Fig-95:** The photomicrograph shows negative staining in outer cells (x400, E-Cadherin).

CASE-9

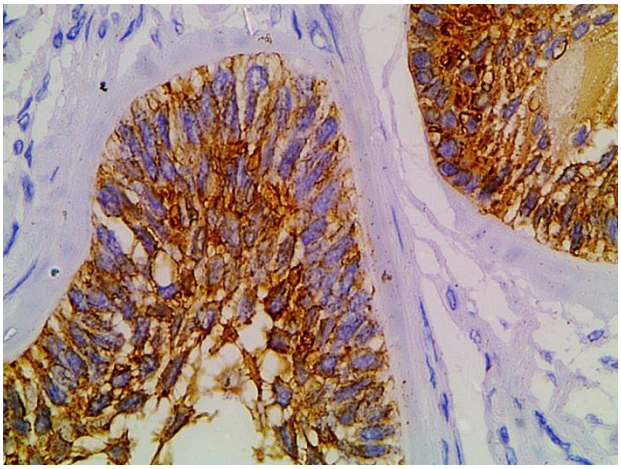
It consists of solid follicular pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell (**Fig-97**). The inner cells are stellate reticulum-like, squamous cells and round cells. The normal equivalent of the outer cells resembles **"Preameloblasts of tooth germ (PA-LBS)."**



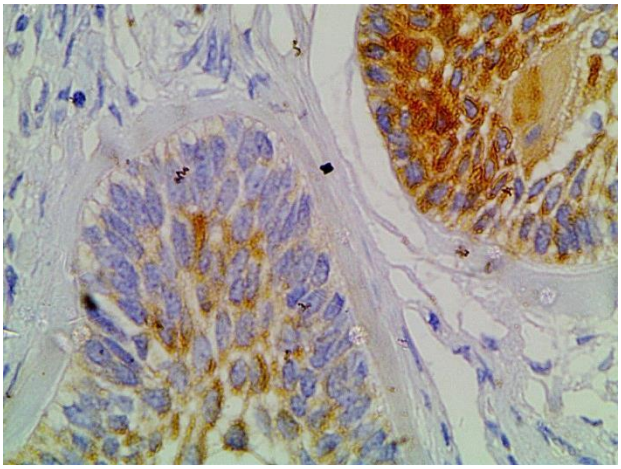
(**Fig-96:** The photomicrograph shows ameloblastoma with outer cells resembling preameloblasts (x100, H&E).



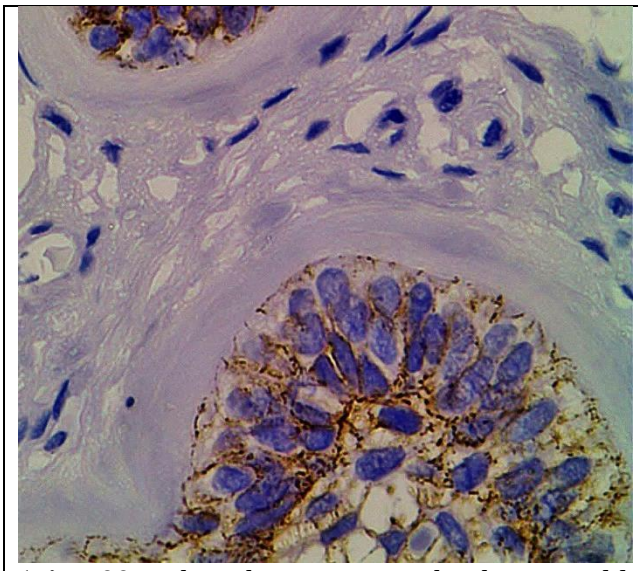
(**Fig-97:** The photomicrograph shows ameloblastoma island with outer cells resembling preameloblasts (x400, H&E).



(**Fig-98:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



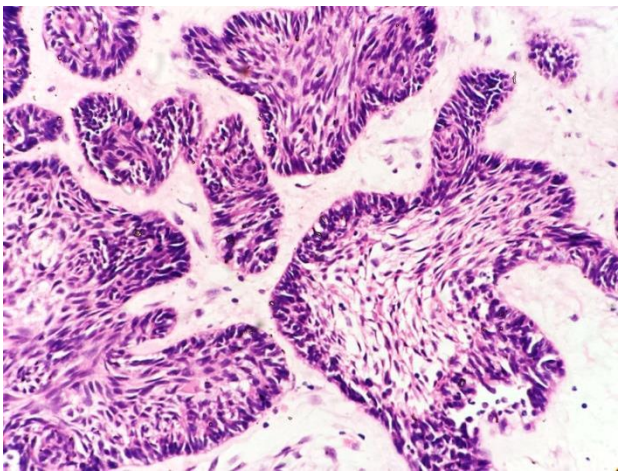
(**Fig-99:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



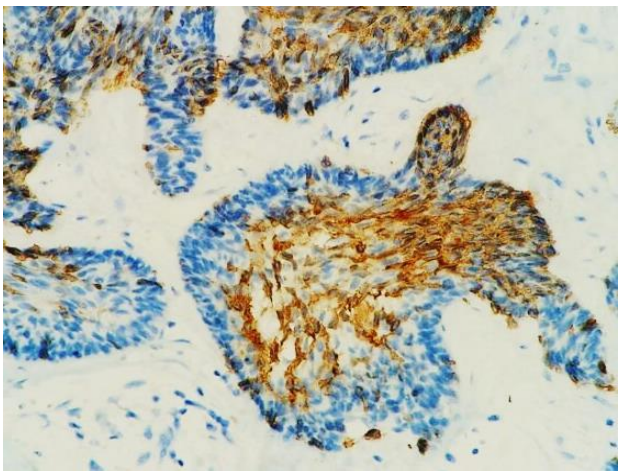
(**Fig-100:** The photomicrograph shows mild staining in membranous areas of outer cells (x400, E-Cadherin).

CASE-10

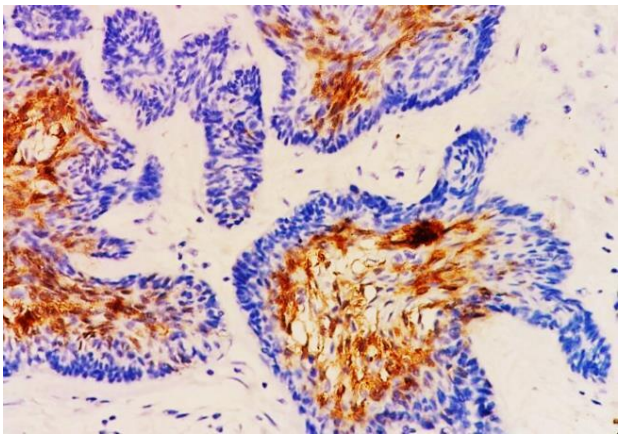
It consists of cystic growth pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell (**Fig-101**). The normal equivalent of the outer cells resembles **"Preameloblasts of tooth germ (PA-LBS)."**



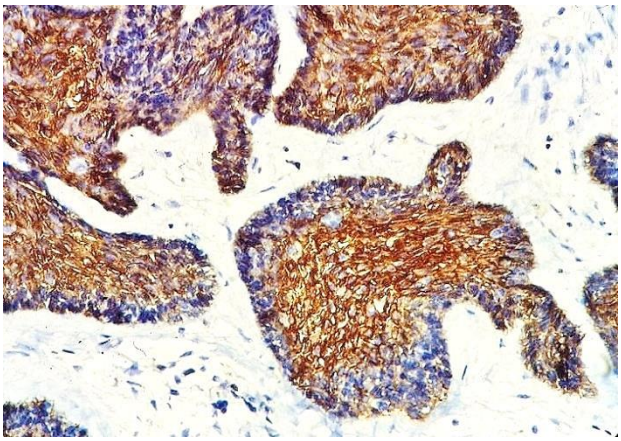
(**Fig-101:** The photomicrograph shows ameloblastoma with outer cells resembling preameloblasts (x400, H&E).



(**Fig-102:** The photomicrograph shows negative staining in outer cells (x400, CK-14).



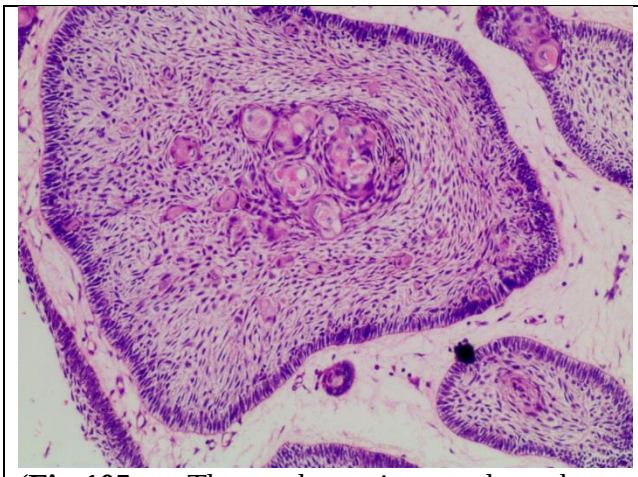
(**Fig-103:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



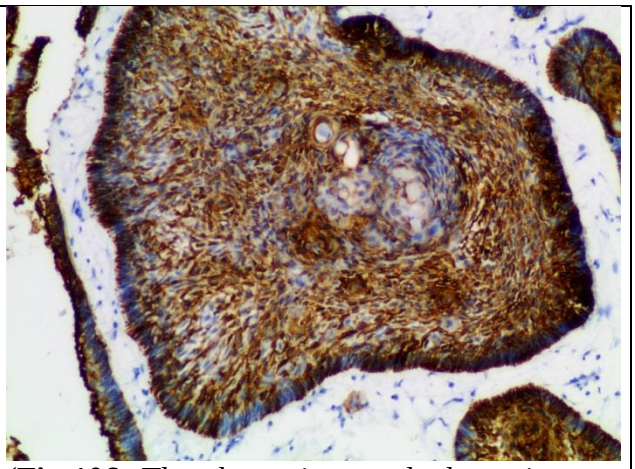
(**Fig-104:** The photomicrograph shows minimal staining in cytoplasmic areas of outer cells (x400, E-Cadherin).

CASE-11

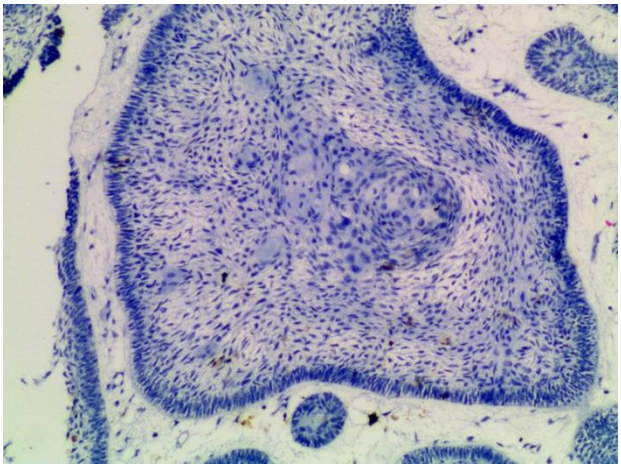
It consists of cystic growth pattern with tall columnar cells containing long and slender nucleus. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell (**Fig-105**). The inner cells are stellate reticulum-like and squamous cells. The normal equivalent of the outer cells resembles **"Preameloblasts as in early bell stage of tooth germ (PA-EBS)."**



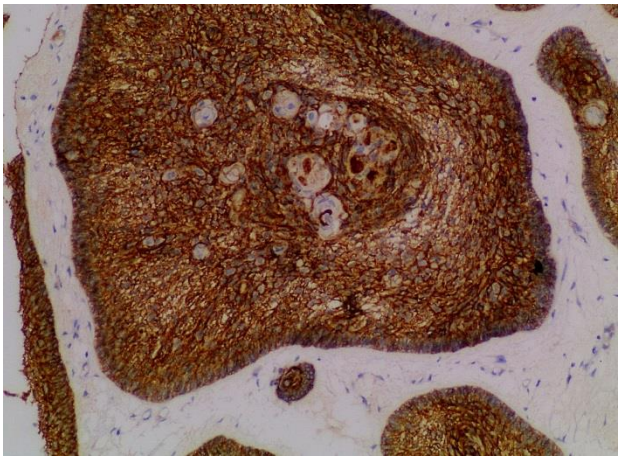
(**Fig-105:** The photomicrograph shows ameloblastoma with outer cells resembling preameloblasts as in early bell stage (x100, H&E).



(**Fig-106:** The photomicrograph shows intense staining in outer cells (x100, CK-14).



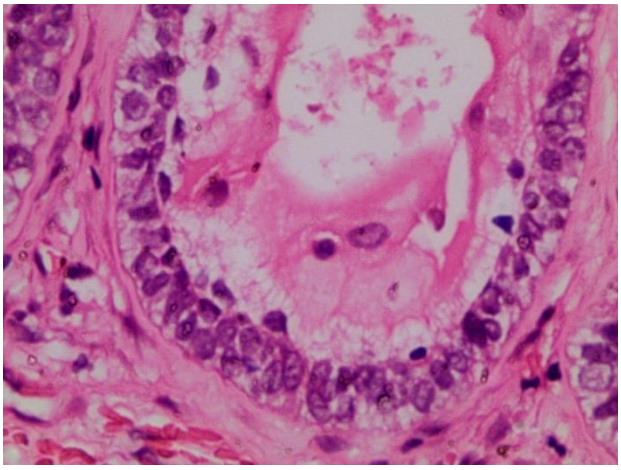
(**Fig-107:** The photomicrograph shows negative staining in outer cells (x100, CK-19).



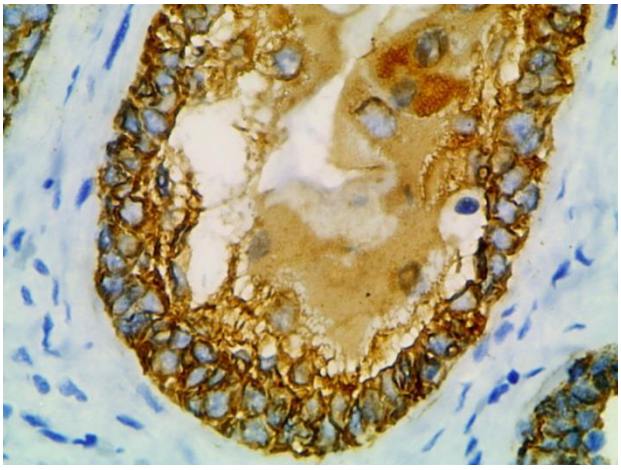
(**Fig-108:** The photomicrograph shows intense staining in cytoplasmic areas of outer cells (x100, E-Cadherin).

CASE-12

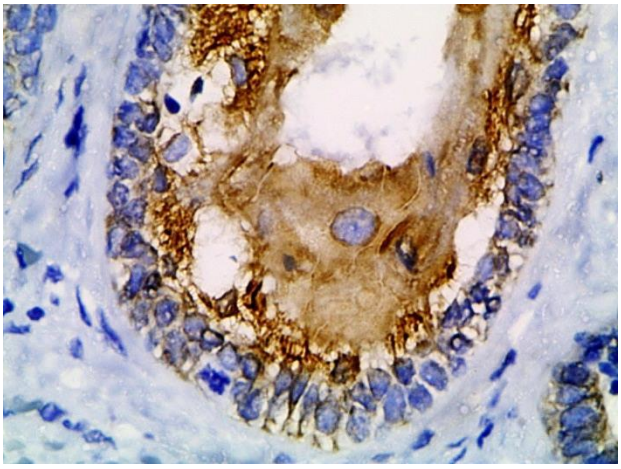
It consists of cystic growth pattern with outer cells are cuboidal or short columnar shaped with round to squared nucleus. The nucleus almost fills the entire cell (**Fig-109**). The inner cells are stellate reticulum-like and keratinizing cells. The normal equivalent of the outer cells resembles **"Inner enamel epithelium of tooth germ (IEE)."**



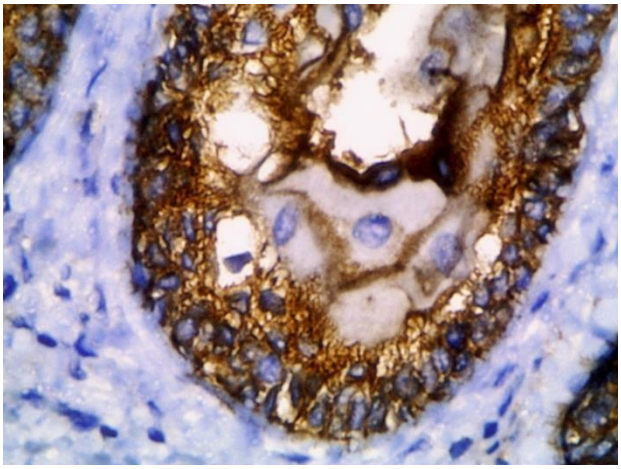
(**Fig-109:** The photomicrograph shows ameloblastoma with outer cells resembling inner enamel epithelium (x400, H&E).



(**Fig-110:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



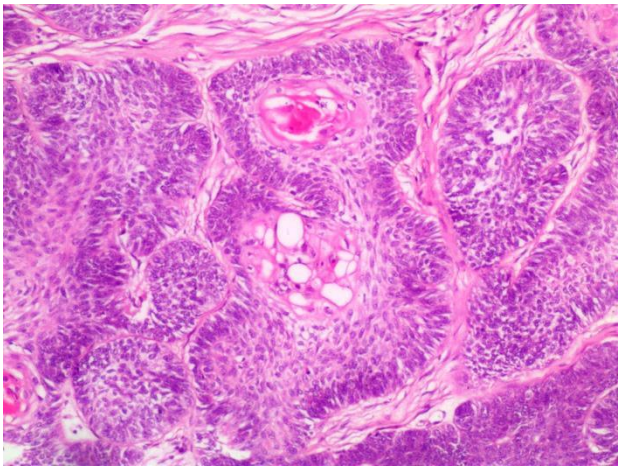
(**Fig-111:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



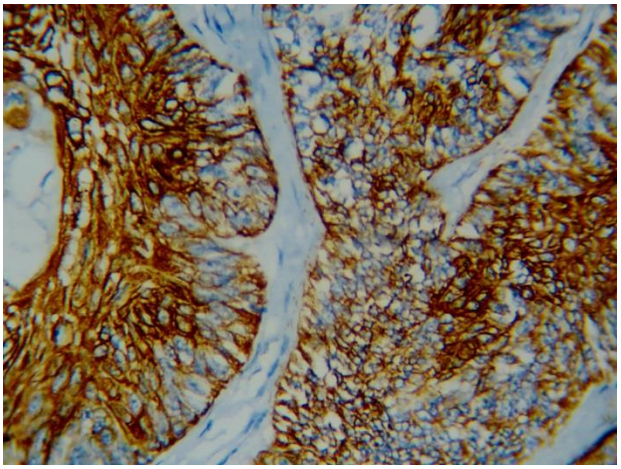
(**Fig-112:** The photomicrograph shows intense staining in cytoplasmic areas of outer cells (x400, E-Cadherin).

CASE-13

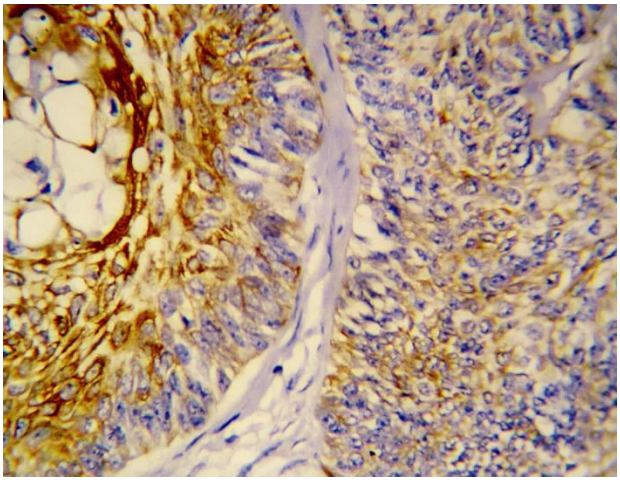
It consists of follicular and plexiform pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell (**Fig-113**). The normal equivalent of the outer cells resembles **"Preameloblasts of tooth germ (PA-LBS)."**



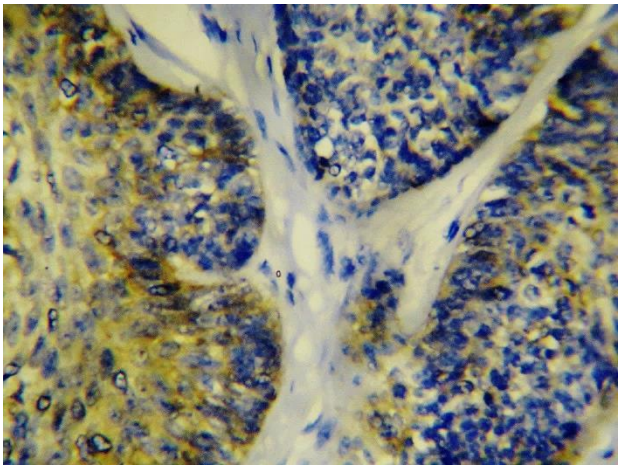
(**Fig-113:** The photomicrograph shows ameloblastoma with outer cells resembling preameloblasts with vacuoles (x100, H&E).



(**Fig-114:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



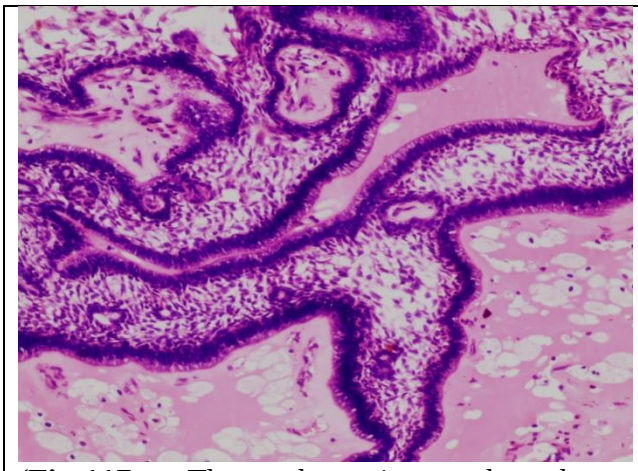
(**Fig-115:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



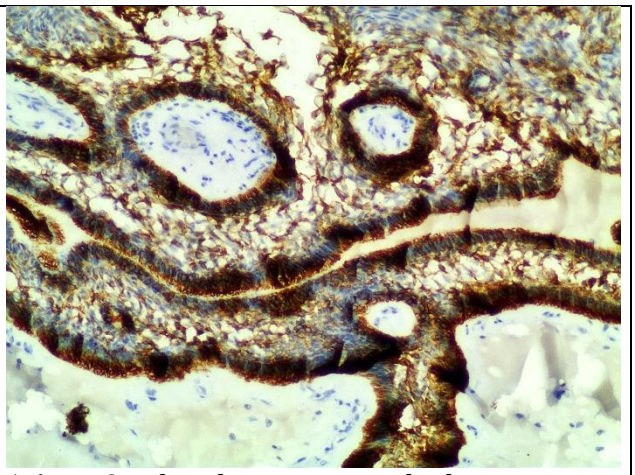
(**Fig-116:** The photomicrograph shows negative staining in outer cells (x100, E-Cadherin).

CASE-14

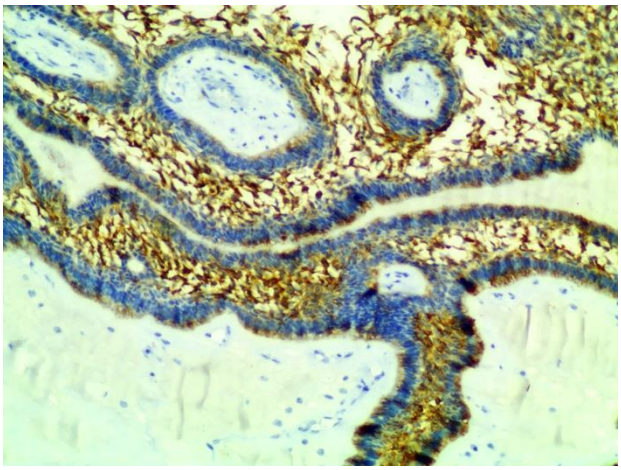
It consists of cystic growth pattern with outer cells are more tall columnar cells containing long and slender nucleus with increased cytoplasmic proportions. The nucleus show reversed polarity and apparent palisading and occupies basal 1/3rd of the cell (**Fig-117**). The inner cells are stellate reticulum-like cells. The normal equivalent of the outer cells resembles **"Presecretory ameloblasts of tooth germ (PSA)."**



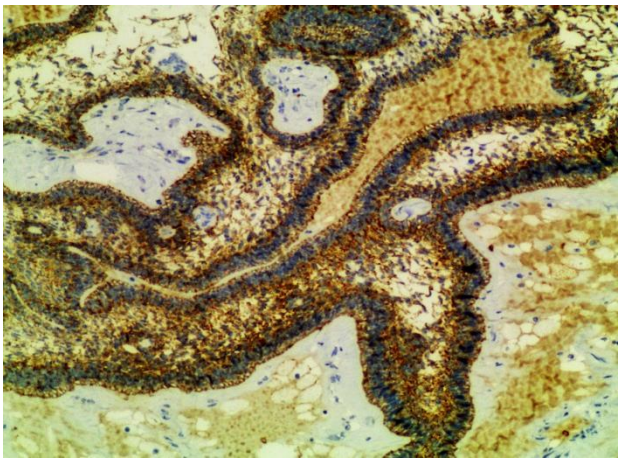
(**Fig-117:** The photomicrograph shows ameloblastoma with outer cells resembling presecretory ameloblasts (x100, H&E).



(**Fig-118**: The photomicrograph shows intense staining in outer cells (x100, CK-14).



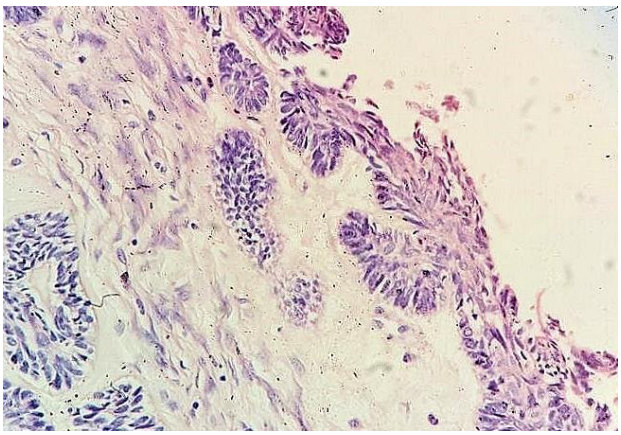
(**Fig-119:** The photomicrograph shows intense staining in more differentiated outer cells (x100, CK-19).



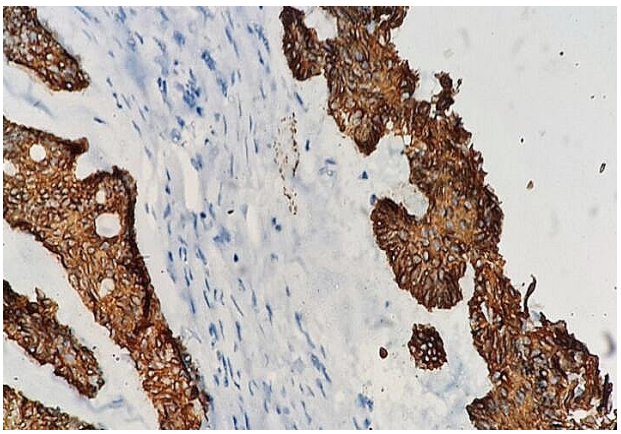
(**Fig-120:** The photomicrograph shows mild staining in outer cells (x100, E-Cadherin).

CASE-15

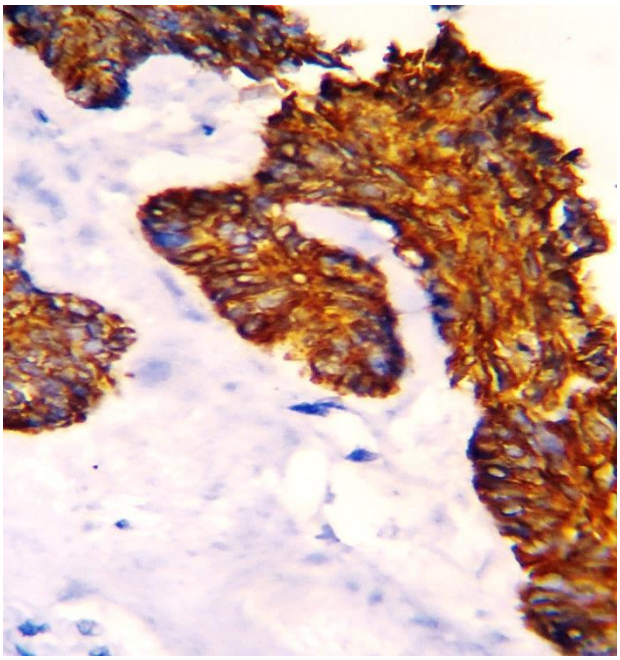
It consists of cystic growth pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell (**Fig-121**). The normal equivalent of the outer cells resembles **"Preameloblasts of tooth germ (PA-LBS)."**



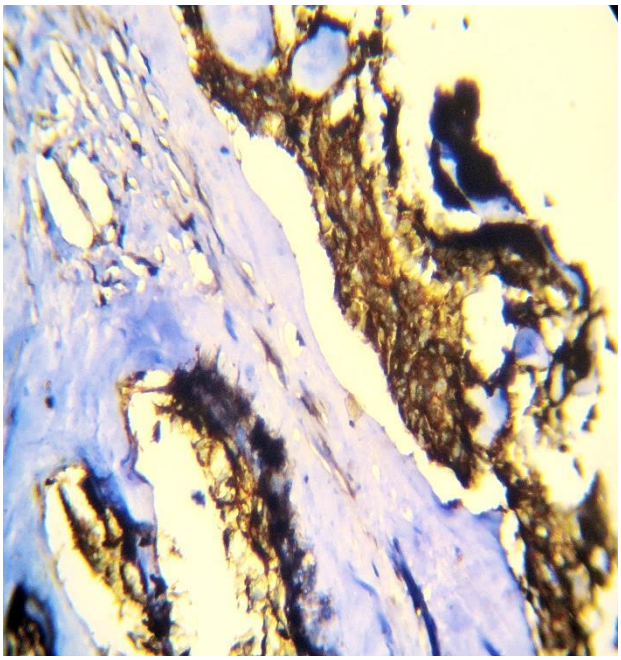
(**Fig-121:** The photomicrograph shows ameloblastoma with outer cells resembling preameloblasts (x400, H&E).



(**Fig-122:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



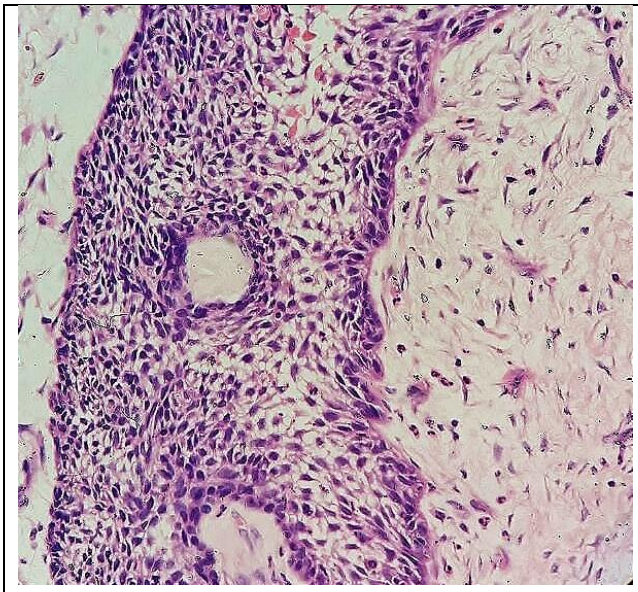
(**Fig-122**: The photomicrograph shows intense staining in outer cells (x400, CK-19).



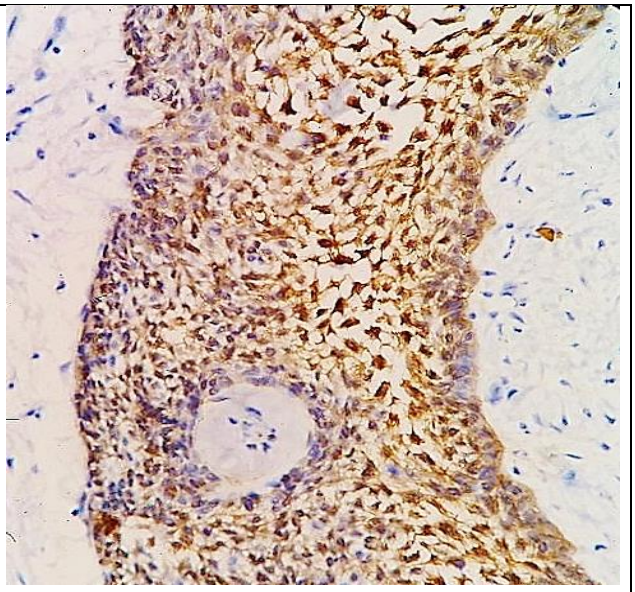
(**Fig-123**: The photomicrograph shows intense staining in cytoplasmic areas of outer cells (x400, E-Cadherin).

CASE-16

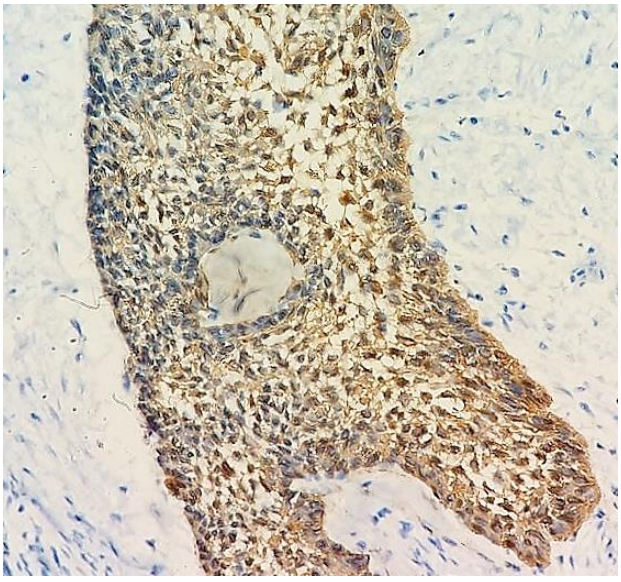
It consists of cystic growth pattern with outer cells of the ameloblastoma are cuboidal cells with centrally placed nucleus. The nucleus almost fills the cell (**Fig-124**). The inner cells are stellate reticulum-like and keratinizing cells. The normal equivalent of the outer cells resembles **"Inner enamel epithelium of tooth germ (IEE)."**



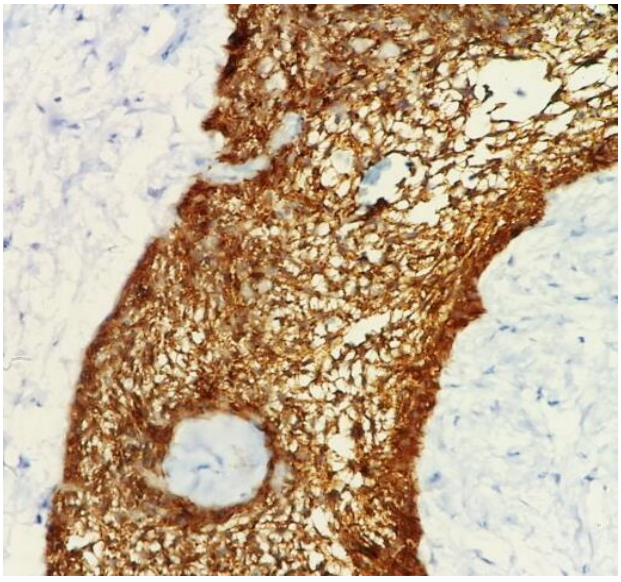
(**Fig-124:** The photomicrograph shows ameloblastoma with outer cells resembling inner enamel epithelium (x400, H&E).



(**Fig-125:** The photomicrograph shows mild staining in outer cells (x400, CK-14).



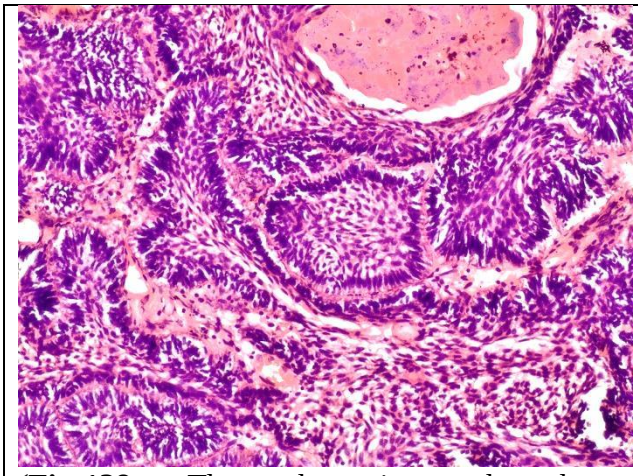
(**Fig-126:** The photomicrograph shows mild staining in outer cells (x400, CK-19).



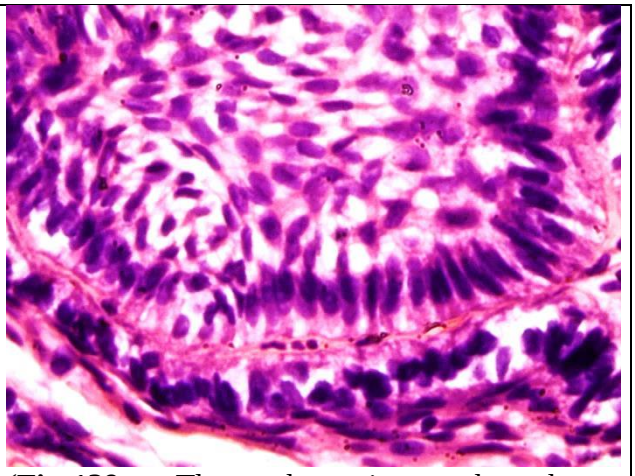
(**Fig-127:** The photomicrograph shows intense staining in cytoplasmic areas of outer cells (x400, E-Cadherin).

CASE-17

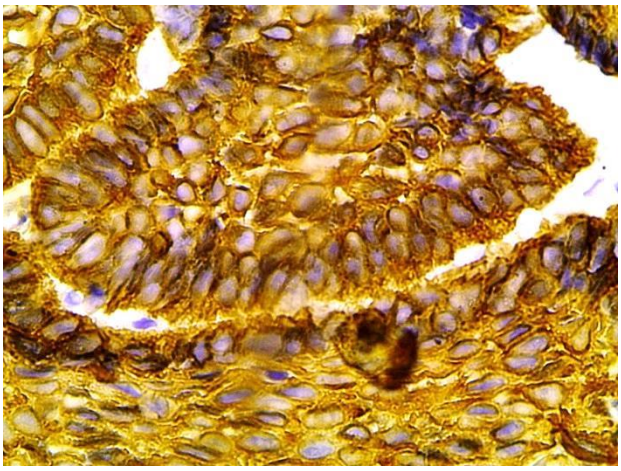
It consists of plexiform pattern with outer cells are more tall columnar cells containing long and slender nucleus with increased cytoplasmic proportions. The nucleus show reversed polarity and apparent palisading and occupies basal 1/3rd of the cell (**Fig-128**). The inner cells are stellate reticulum-like and round cells. The normal equivalent of the outer cells resembles **"Presecretory ameloblasts of tooth germ (PSA)."**



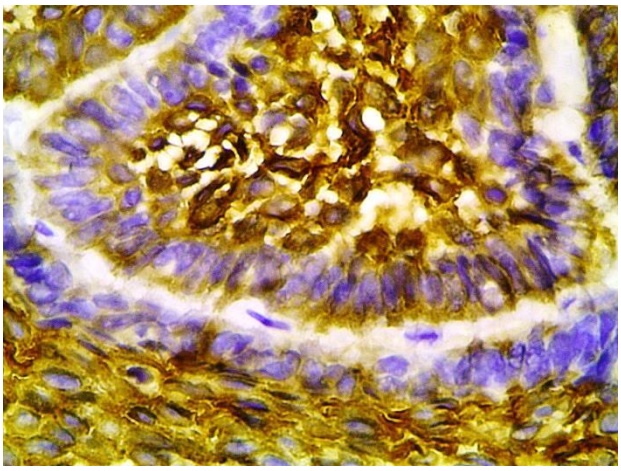
(**Fig-128:** The photomicrograph shows ameloblastoma with outer cells resembling presecretory ameloblasts and inner stellate reticulum like cells (x100, H&E).



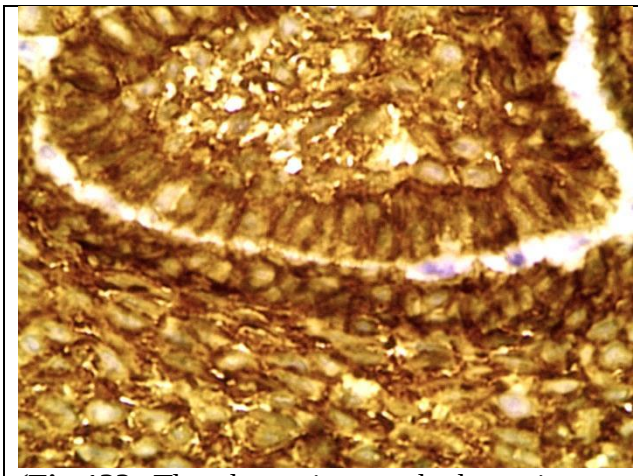
(**Fig-129:** The photomicrograph shows ameloblastoma with outer cells resembling presecretory ameloblasts (x400, H&E).



(**Fig-130:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



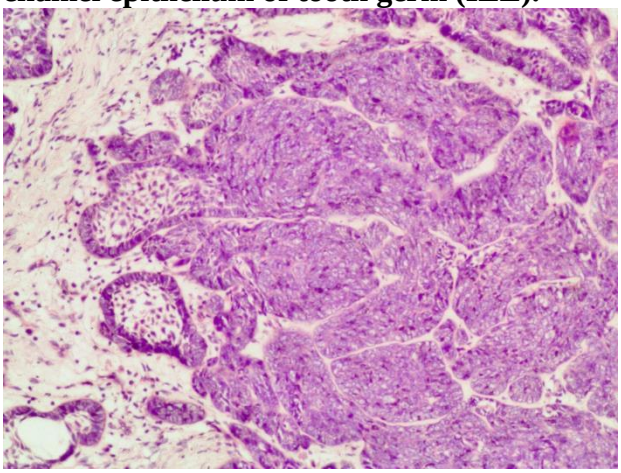
(**Fig-131:** The photomicrograph shows intense staining in more differentiated outer cells (x400, CK-19).



(**Fig-132:** The photomicrograph shows intense staining in the cytoplasmic areas of outer cells (x400, E-Cadherin).

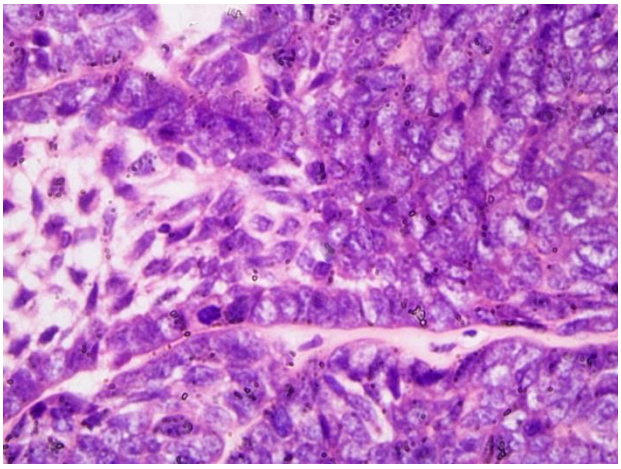
CASE-18

It consists of follicular, plexiform and basaloid pattern with outer cells cuboidal or short columnar shaped with round to squared nucleus. The nucleus almost fills the entire cell. The inner cells are stellate reticulum-like and round cells (**Fig-134**). The normal equivalent of the outer cells resembles **"Inner enamel epithelium of tooth germ (IEE)."**

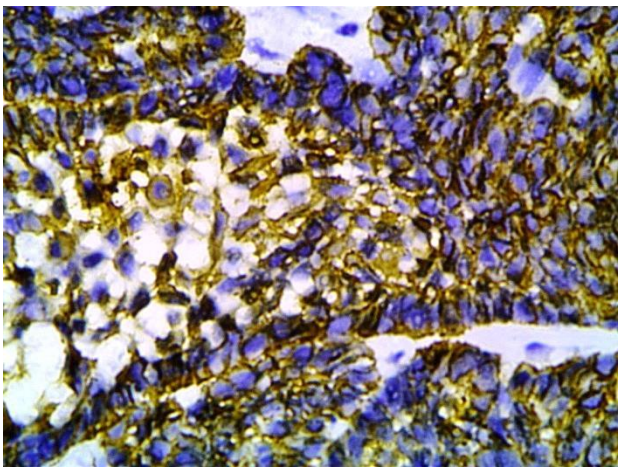


(**Fig-133:** The photomicrograph shows ameloblastoma with outer cells resembling inner enamel epithelium in some areas and adjacent cells are unclassifiable (x100, H&E).

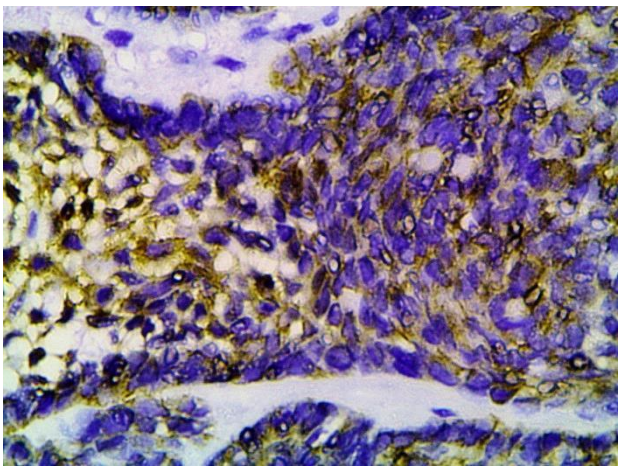
The other outer cells are round nucleated cells without definable cytoplasm. The inner cells are stellate reticulum-like and round cells. The outer cells cannot be compared with any normal cell of developing tooth germ. **"Unclassifiable"**



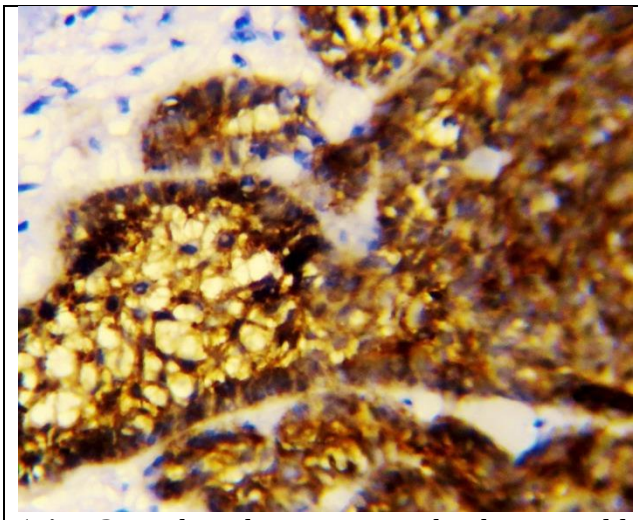
(**Fig-134:** The photomicrograph shows ameloblastoma with outer cells resembling inner enamel epithelium in some areas (x400, H&E).



(**Fig-135:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



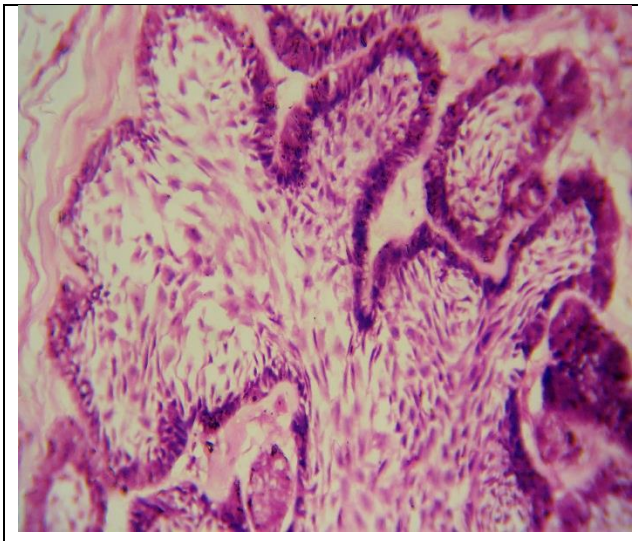
(**Fig-136:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



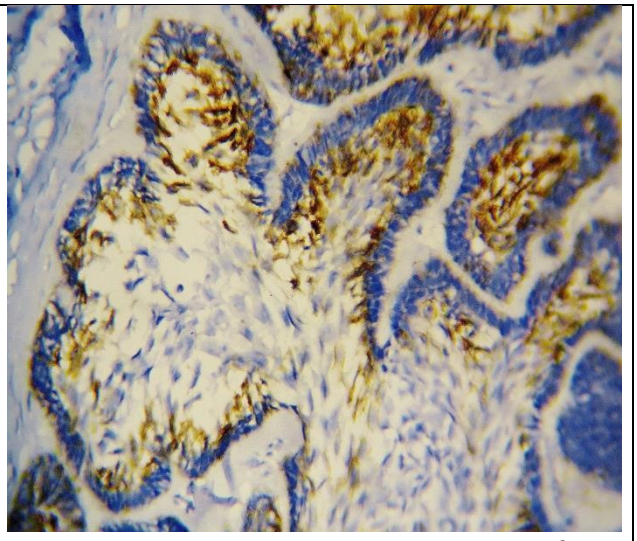
(**Fig-137**: The photomicrograph shows mild staining in cytoplasmic areas of outer cells (x400, E-Cadherin).

CASE-19

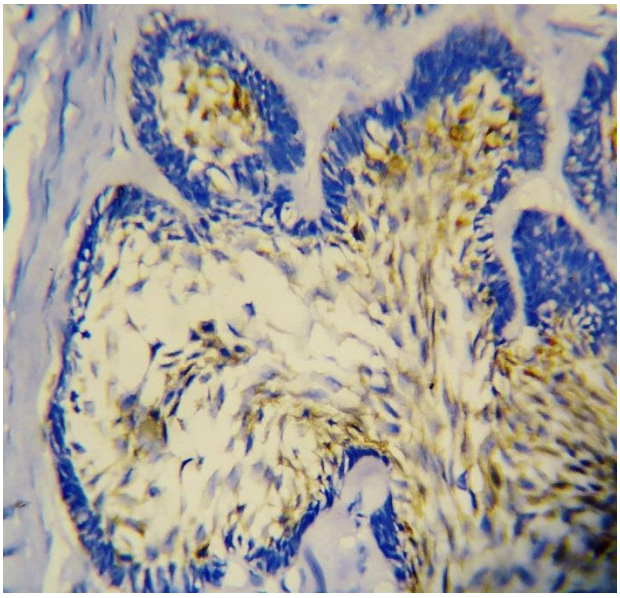
It consists of follicular pattern with outer cells are columnar cells containing oval and elongated nucleus. The nucleus show reversed polarity with apparent pseudostratification and occupies almost half of the cell (**Fig-138**). The inner cells are stellate reticulum-like cells. The normal equivalent of the outer cells resembles **"Preameloblasts of tooth germ (PA-LBS)."**



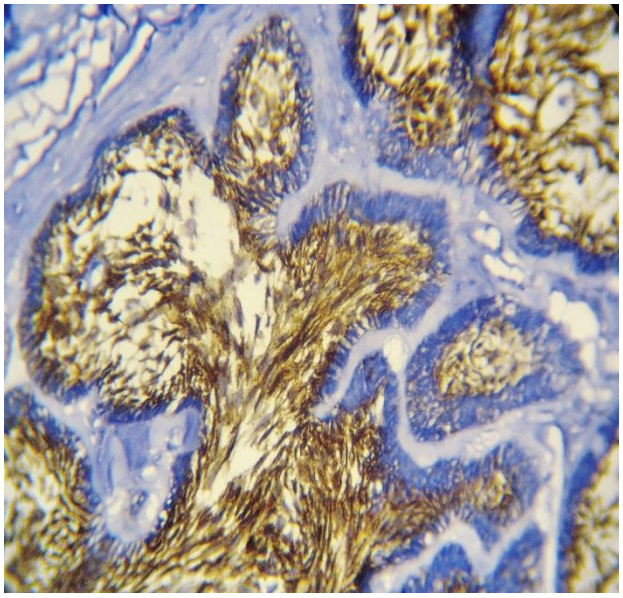
(**Fig-138:** The photomicrograph shows ameloblastoma with outer cells resembling preameloblasts (x400, H&E).



(**Fig-139:** The photomicrograph shows focal staining in inner cells but not the outer cells (x400, CK-14).



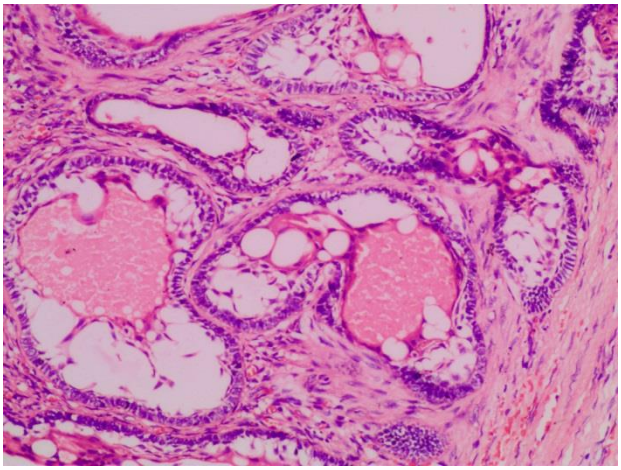
(**Fig-140:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



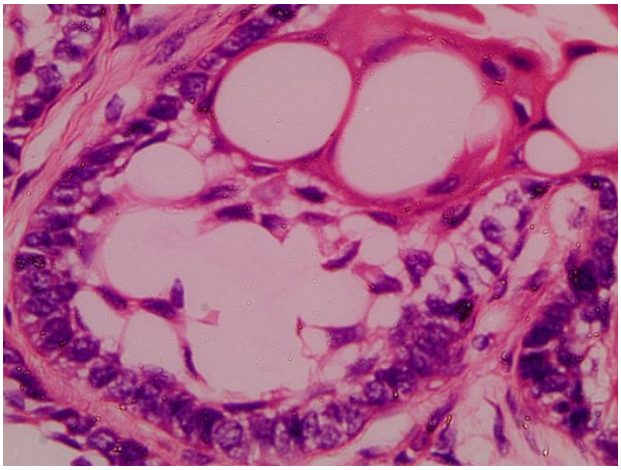
(**Fig-141:** The photomicrograph shows negative staining in outer cells (x400, E-Cadherin).

CASE-20

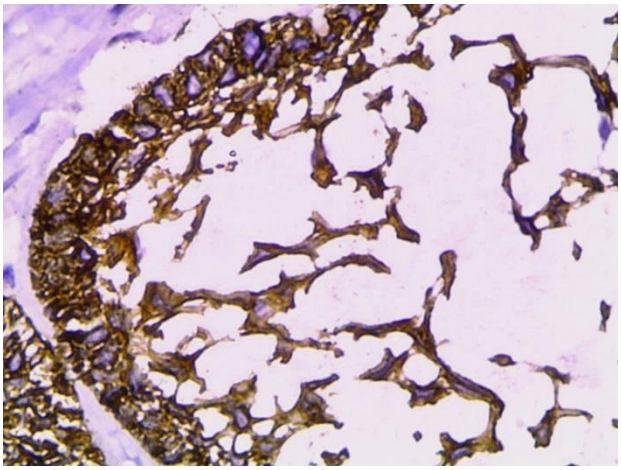
It consists of follicular pattern with outer cells cuboidal or short columnar shaped with round to oval nucleus. The nucleus almost fills the entire cell (**Fig-143**). The inner cells are stellate reticulum-like and acanthomatous cells. The normal equivalent of the outer cells resembles **"Inner enamel epithelium of tooth germ (IEE)."**



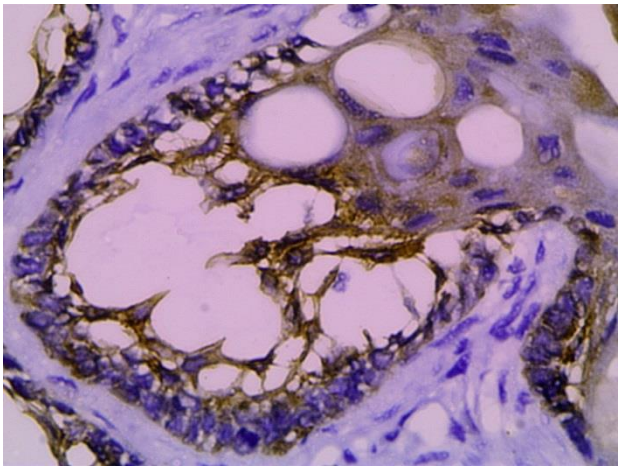
(**Fig-142:** The photomicrograph shows ameloblastoma with outer cells resembling inner enamel epithelium (x100, H&E).



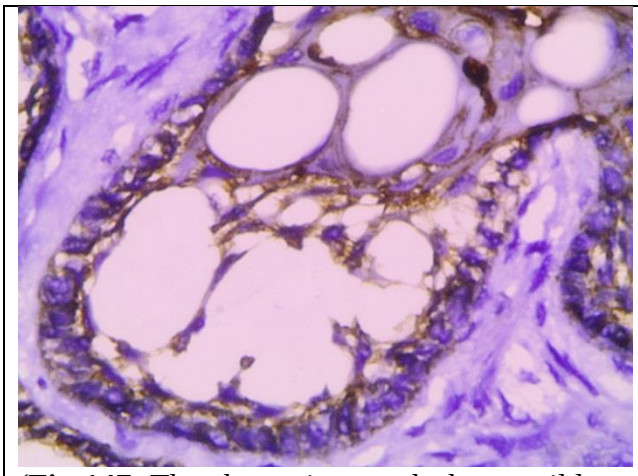
(**Fig-143:** The photomicrograph shows ameloblastoma with outer cells resembling inner enamel epithelium (x400, H&E).



(**Fig-145:** The photomicrograph shows intense staining in outer cells (x400, CK-14).



(**Fig-146:** The photomicrograph shows negative staining in outer cells (x400, CK-19).



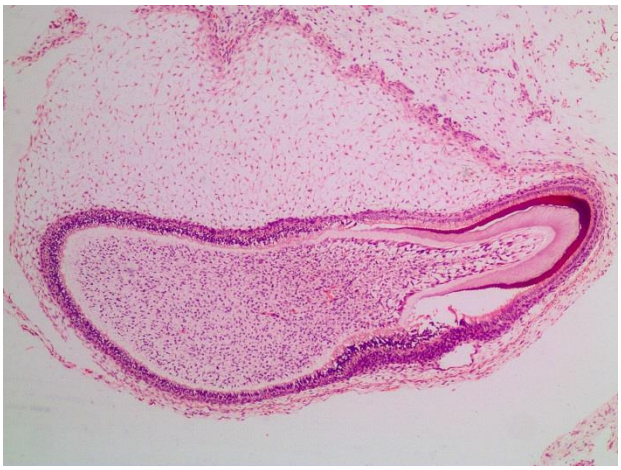
(**Fig-147:** The photomicrograph shows mild staining in cytoplasmic areas of outer cells (x400, E-Cadherin).

Chart-IV: Results of expression patterns of CK-14, CK-19 and E-Cadherin in tooth germ

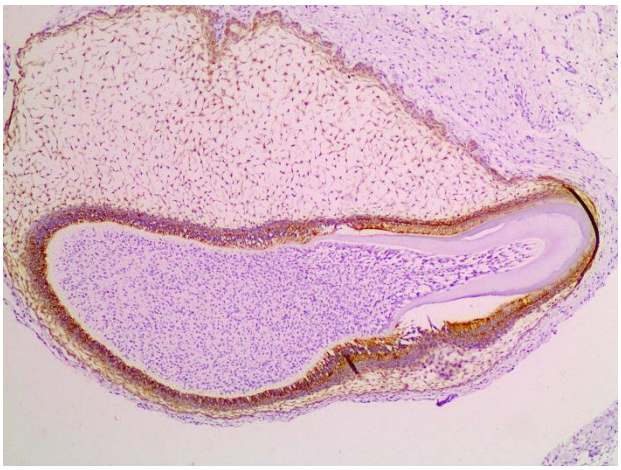
TYPE OF CELLS	Tooth germ evaluated	CK-14	CK-19	E-Cadherin	
Inner enamel epithelium (IEE)	LBS-1	P++	N	N	
	LBS-2	P++	N	N	
	LBS-3	P++	N	P+ (C)	
	LBS-4	P++	N	P++(C)	cervical loop negative for Ck19
	LBS-5	P++	N	P+	
	LBS-6	P++	N	N	
	EBS-1	P++	N	N	
	EBS-2	P++	N	P+	
Preameloblasts (PA-EBS, PA-LBS)	LBS-1	P++	P+	P+(C)	
	LBS-2	P++	P++	P+(C)	
	LBS-3	P++	P++	N	
	LBS-4	P++	P++	P++(C)	
	LBS-5	P++	P+	N	
	LBS-6	P++	N	N=P	
	EBS-1	P++	P+	P+	
	EBS-2	P++	P+	P+	
Presecretory ameloblasts (PSA)	LBS-1	P++	P++	P+(C)	
	LBS-2	P++	P++	P+(C)	
	LBS-3	P++	P+	N	
	LBS-4	P++	P+	N	
	LBS-5	P++	N	N	
	LBS-6	P++	N	N	
Secretory ameloblasts (SA)	LBS-1	P++	P++	N	
	LBS-2	P++	P++	N	
	LBS-3	P++	P++	N	
	LBS-4	P++	P++	P+(C)	
	LBS-5	P++	P++	P+ (C)	
	LBS-6	P+	N	N	

CHART-V: Photographic illustration of tooth germ with expression pattern

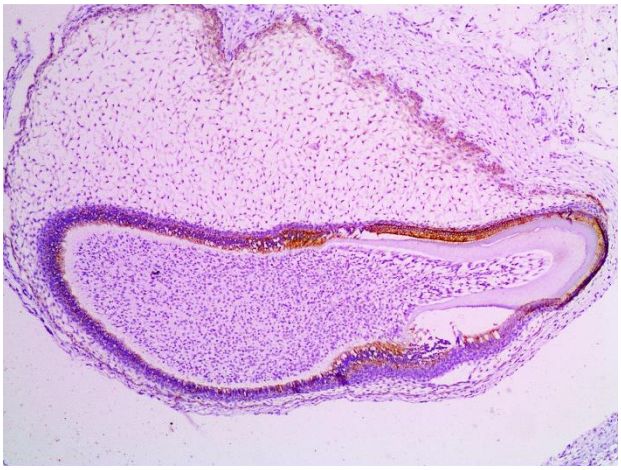
Late bell stage-1



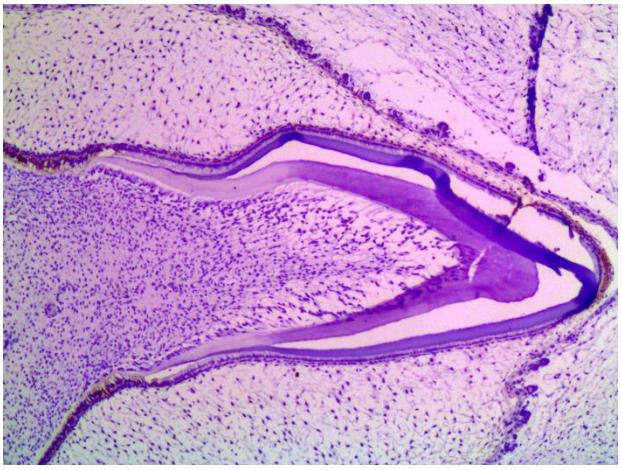
(**Fig-152:** The photomicrograph shows tooth germ of a deciduous maxillary incisor at late bell stage (x40, H&E).



(**Fig-153:** The photomicrograph shows tooth germ of a deciduous maxillary incisor at late bell stage with immunostaining (x40, CK-14).

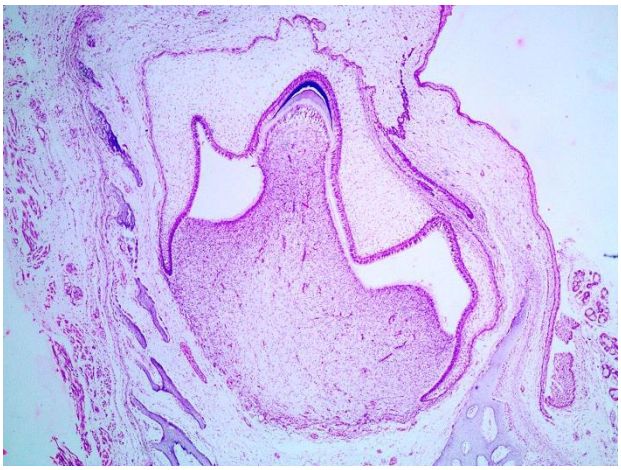


(**Fig-154:** The photomicrograph shows tooth germ of a deciduous maxillary incisor at late bell stage with immunostaining (x40, CK19).

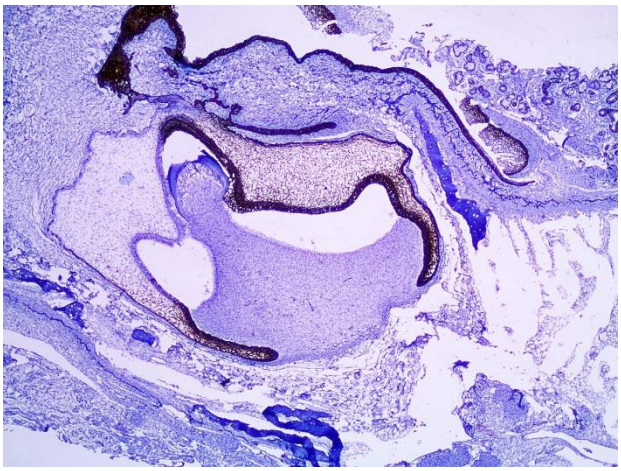


(**Fig-155:** The photomicrograph shows tooth germ of a deciduous maxillary incisor at late bell stage with immunostaining (x40, E-Cadherin).

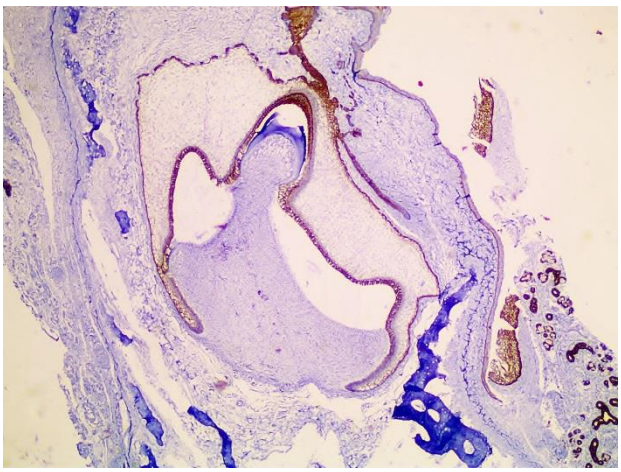
Late bell stage-3



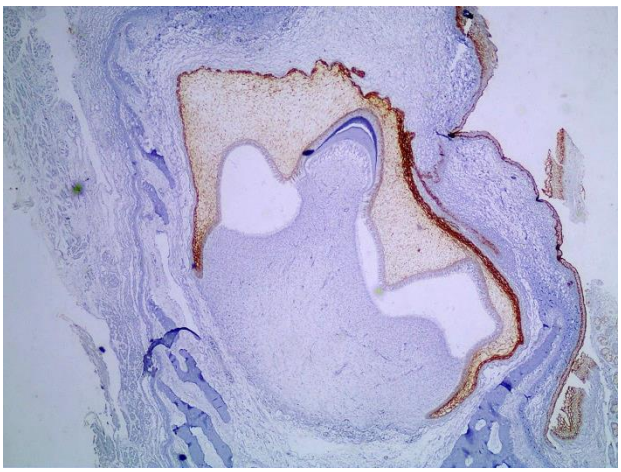
(**Fig-156:** The photomicrograph shows tooth germ of a deciduous mandibular molar in late bell stage (x20, H&E).



(**Fig-157:** The photomicrograph shows tooth germ of a deciduous mandibular molar in late bell stage with immunostaining (x20, CK-14).

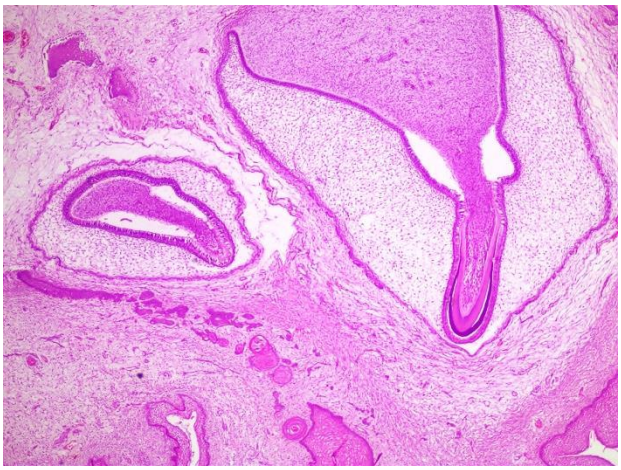


(**Fig-158:** The photomicrograph shows tooth germ of a deciduous mandibular molar in late bell stage with immunostaining (x20, CK-19).

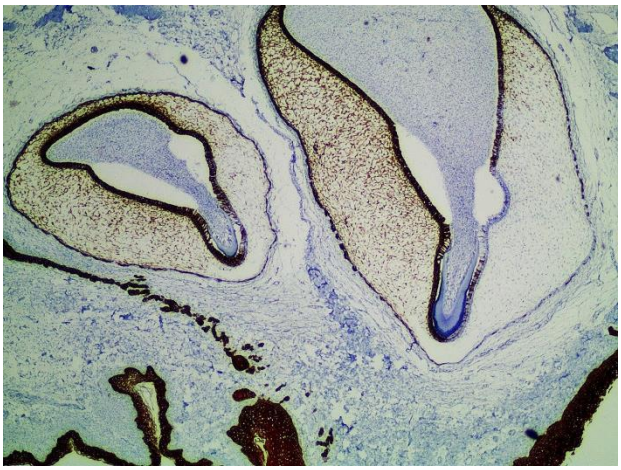


(**Fig-159:** The photomicrograph shows tooth germ of a deciduous mandibular molar in late bell stage with immunostaining (x20, E-Cadherin).

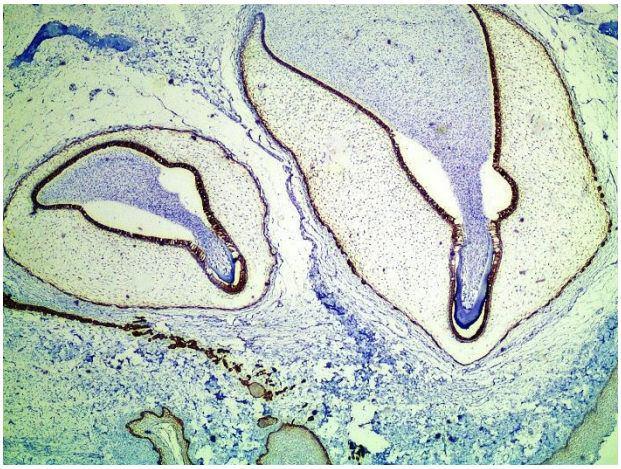
Late bell stage-4



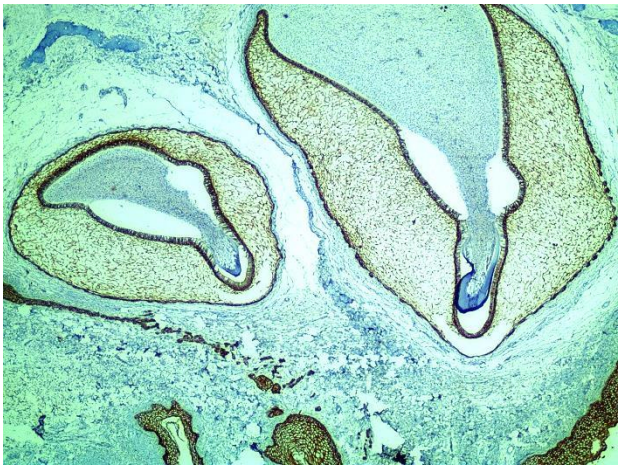
(**Fig-148:** The photomicrograph shows tooth germ of deciduous maxillary incisors at late bell stage (x20, H&E).



(**Fig-149:** The photomicrograph shows tooth germ of deciduous maxillary incisors at late bell stage with immunostaining (x20, CK-14).

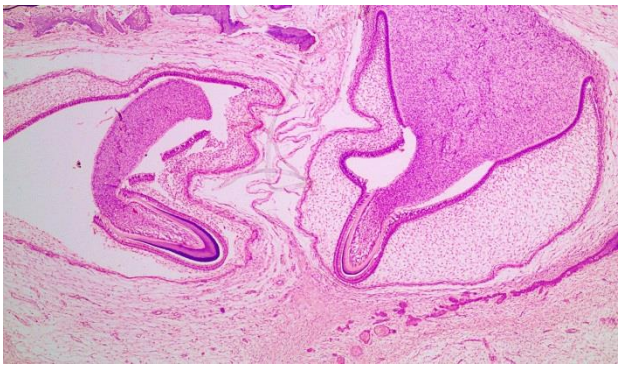


(**Fig-150:** The photomicrograph shows tooth germ of deciduous maxillary incisors at late bell stage with immunostaining (x20, CK-19).

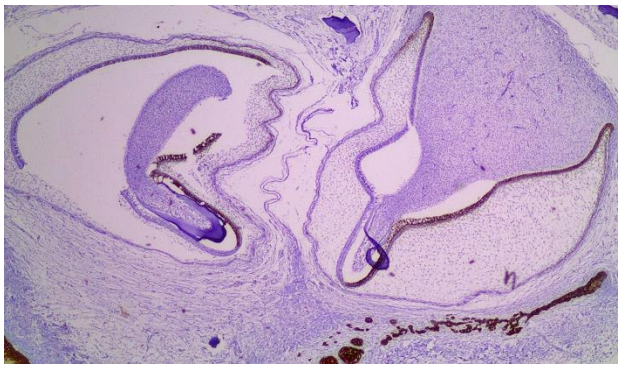


(**Fig-151:** The photomicrograph shows tooth germ of deciduous maxillary incisors at late bell stage with immunostaining (x20, E-Cadherin).

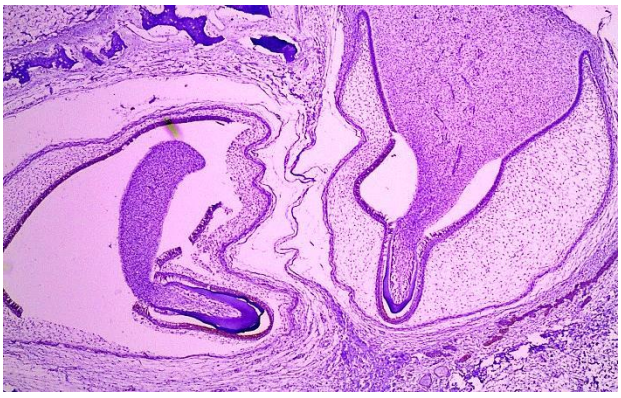
Late bell stage-5



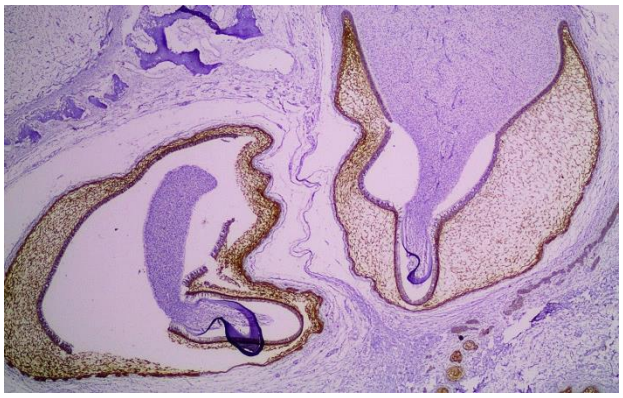
(**Fig-160:** The photomicrograph shows tooth germ of deciduous maxillary incisors in late bell stage (x20, H&E).



(**Fig-161:** The photomicrograph shows tooth germ of deciduous maxillary incisors in late bell stage with immunostaining (x20, CK-14).



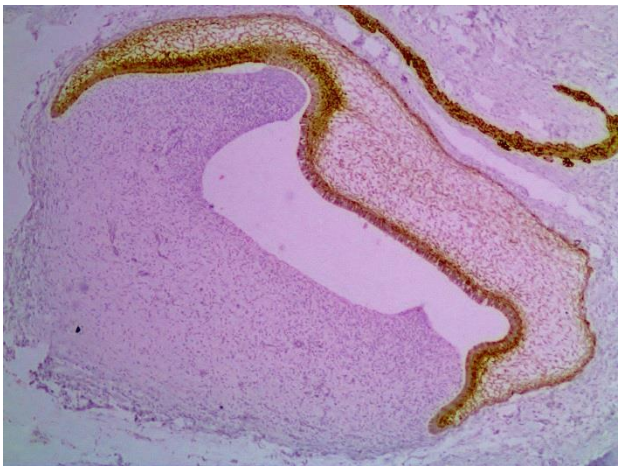
(**Fig-162:** The photomicrograph shows tooth germ of deciduous maxillary incisors in late bell stage with immunostaining (x20, CK-19).



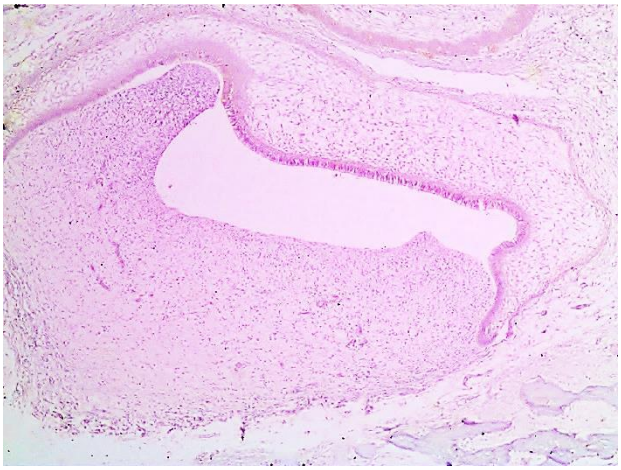
(**Fig-163:** The photomicrograph shows tooth germ of deciduous maxillary incisors in late bell stage with immunostaining (x20, E-Cadherin).

Early bell stage-1

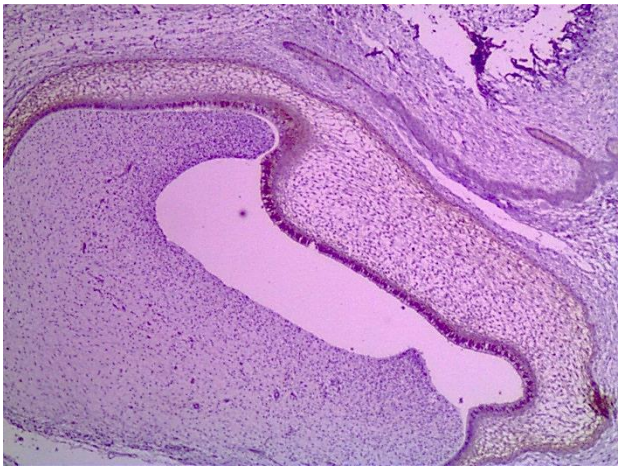
(**Fig-164:** The photomicrograph shows mandibular molar tooth germ in early bell stage (x40, H&E).



(**Fig-165:** The photomicrograph shows mandibular molar tooth germ in early bell stage with immunostaining (x40, CK14).

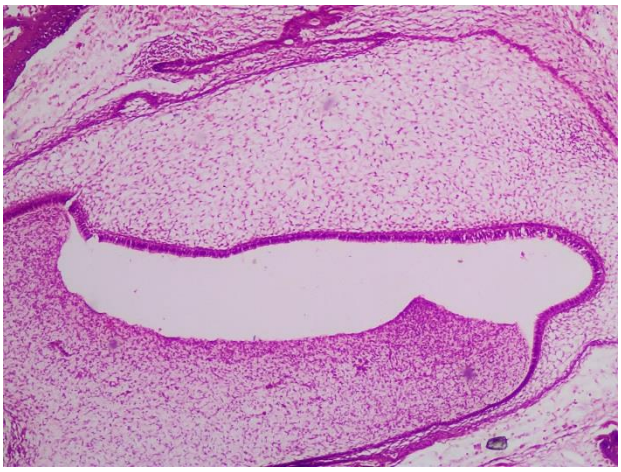


(**Fig-166:** The photomicrograph shows mandibular molar tooth germ in early bell stage with immunostaining (x40, CK19).

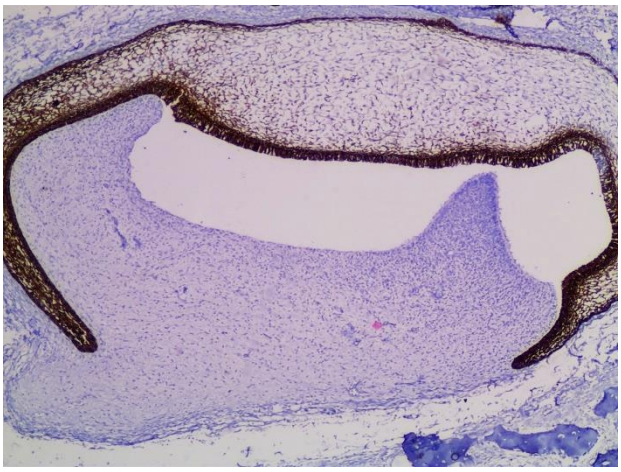


(**Fig-167:** The photomicrograph shows mandibular molar tooth germ in early bell stage with immunostaining (x40, E-Cadherin).

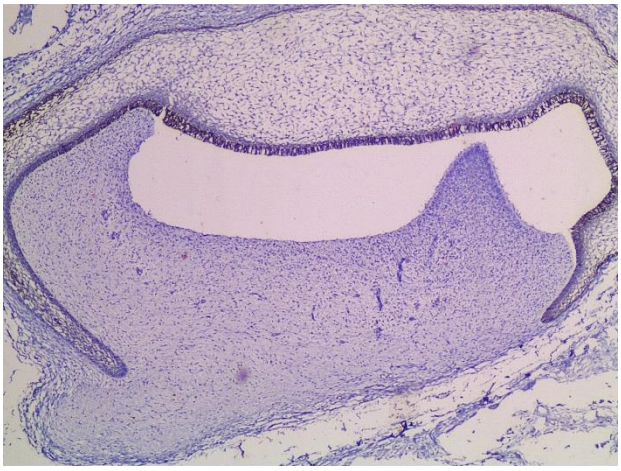
Early bell stage-2



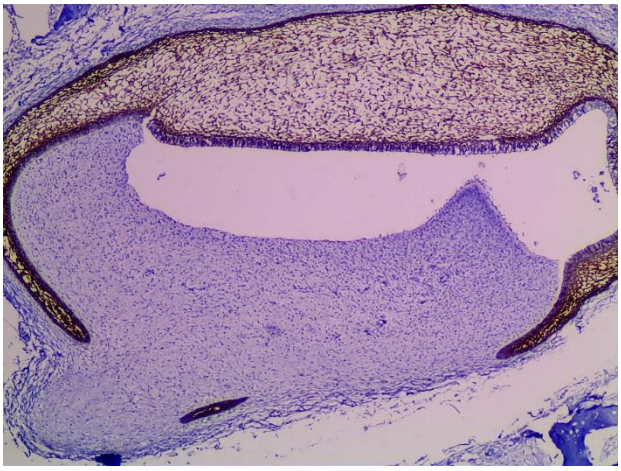
(**Fig-168:** The photomicrograph shows mandibular molar tooth germ in early bell stage (x40, H&E).



(Fig-169: The photomicrograph shows mandibular molar tooth germ in early bell stage with immunostaining (x40, CK-14).



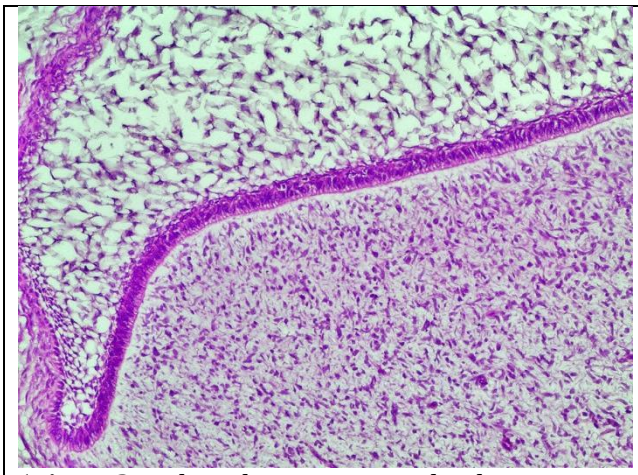
(**Fig-170:** The photomicrograph shows mandibular molar tooth germ in early bell stage with immunostaining (x40, CK-19).



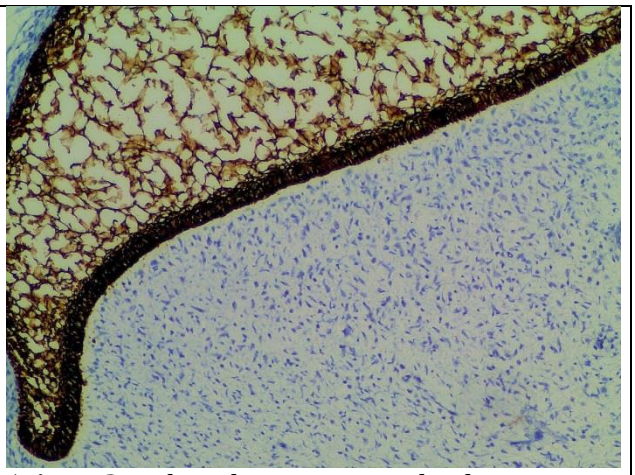
(**Fig-171:** The photomicrograph shows mandibular molar tooth germ in early bell stage with immunostaining (x40, E-Cadherin).

INNER ENAMEL EPITHELIUM

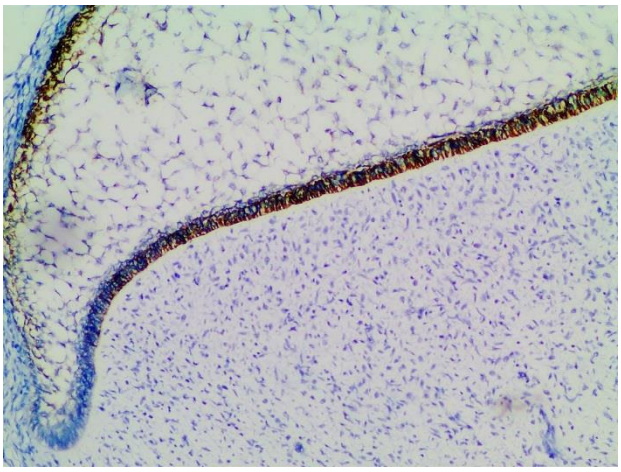
The zone of inner enamel epithelial cells extends from the tip of the cervical loop to the point of transition of preameloblasts. These cells are columnar shaped cells containing round to oval shaped nucleus, which is arranged at different levels (predominantly central zone) and occupies almost the entire cell. A distinct basement membrane zone is visible. The cells are bordered by undifferentiated ectomesenchymal cells of the dental papilla on one side and stellate reticulum cells on the other side with an acellular but fibrillar zone is interposed between them (**Fig-172**).



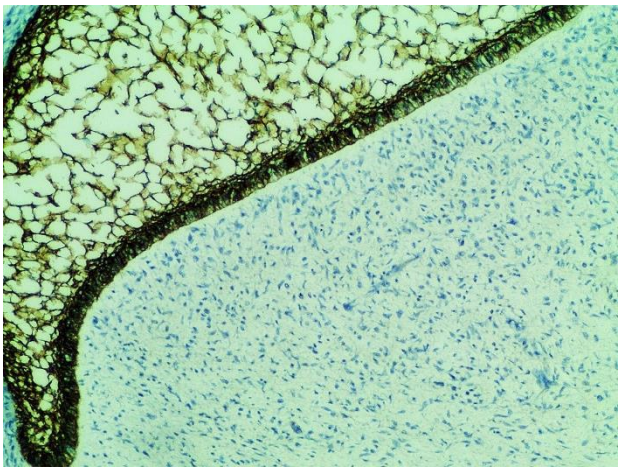
(**Fig-172:** The photomicrograph shows inner enamel epithelium (x100, H&E).



(**Fig-173:** The photomicrograph shows inner enamel epithelium with intense immunostaining (x100, CK-14).



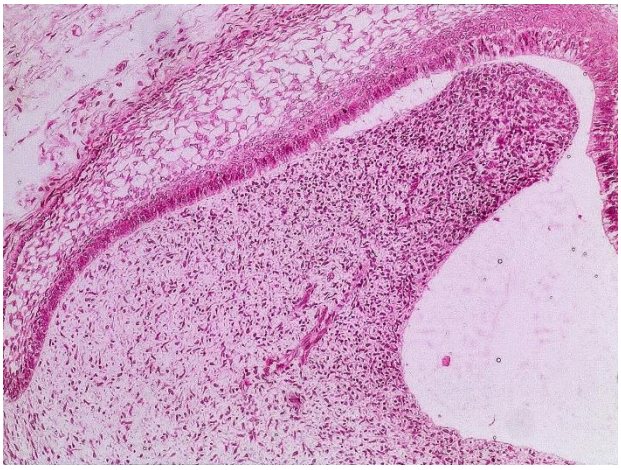
(**Fig-174:** The photomicrograph shows immunostaining in the preameloblasts but negative in inner enamel epithelium, which is present in the cervical loop (x100, CK-19).



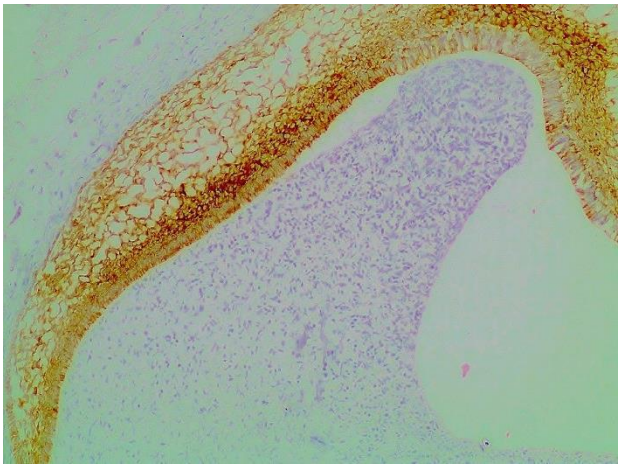
(**Fig-175:** The photomicrograph shows inner enamel epithelium with intense immunostaining (x100, E-cadherin).

PREAMELOBLASTS OF EARLY BELL STAGE

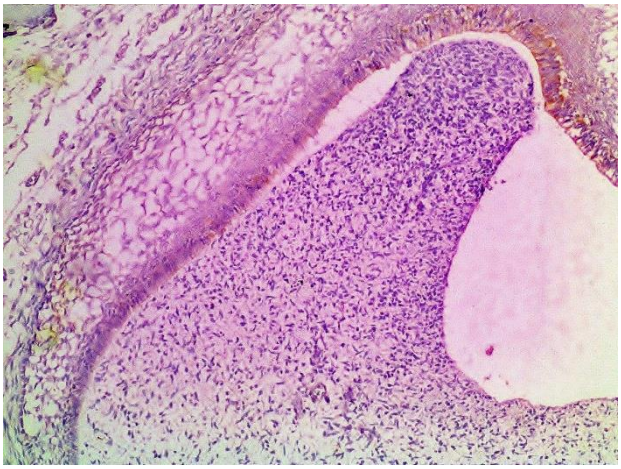
The zone of preameloblast cells extends from the transition of inner enamel epithelium to the slopes of the cusp tip. It shows gradual differentiation and the cells are tall columnar shaped cells containing oval to long and slender nucleus especially at the cuspal slopes. The nucleus show reversal of polarity, but appears pseudostratified and occupies almost half of the cell. The cells are bordered by undifferentiated but condensed ectomesenchymal cells of the dental papilla on one side and stratum intermedium cells on the other side. The acellular zone between them is less distinct.



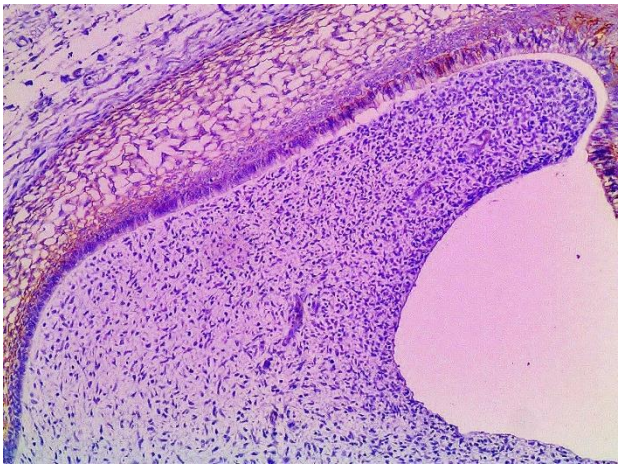
(**Fig-188:** The photomicrograph shows preameloblasts of early bell stage (x40, H&E).



(**Fig-189:** The photomicrograph shows preameloblasts of early bell stage with intense staining (x40, CK-14).



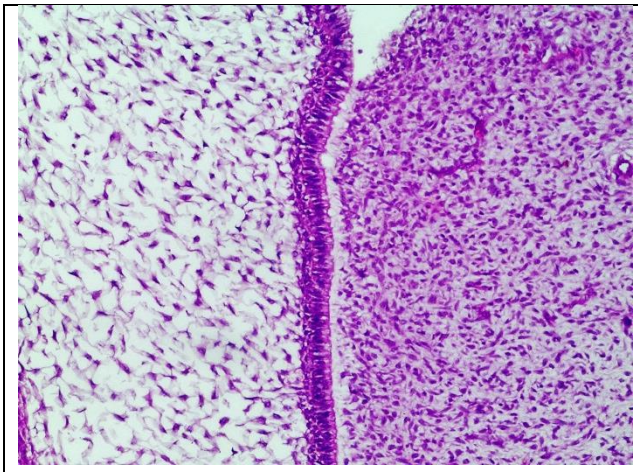
(**Fig-190:** The photomicrograph shows preameloblasts of early bell stage with mild staining (x40, CK-19).



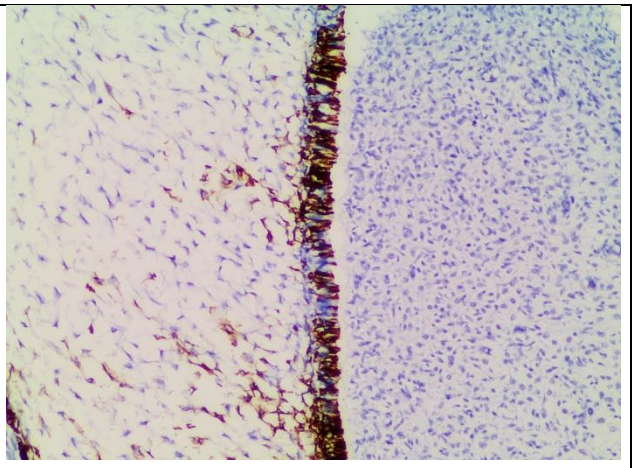
(**Fig-191:** The photomicrograph shows preameloblasts of early bell stage with mild staining (x40, E-Cadherin).

PREAMELOBLASTS OF LATE BELL STAGE

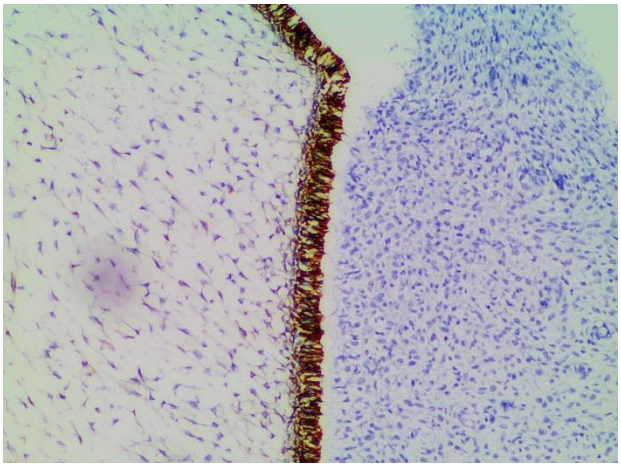
The zone of preameloblast cells extends from the transition point of inner enamel epithelium to the transition point of presecretory ameloblasts. The cells are tall columnar shaped cells containing oval shaped, elongated, hyperchromatic nucleus. The nucleus shows reversed polarity with apparent pseudostratification (but no overt palisading) and occupies almost half of the cell. The cells are bordered by undifferentiated but condensed ectomesenchymal cells of the dental papilla on one side and stratum intermedium cells on the other side. There is no evidence of hard tissue formation and an acellular zone intervenes between the preameloblast layer and dental papilla with an intact basement membrane (**Fig-176**).



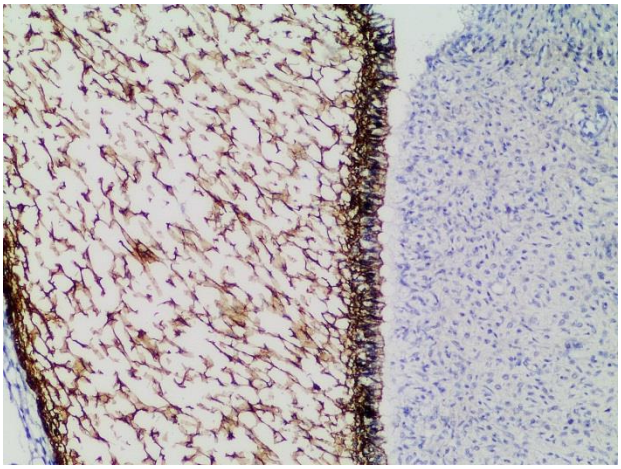
(**Fig-176:** The photomicrograph shows preameloblasts (x100, H&E).



(**Fig-177:** The photomicrograph shows intense staining in preameloblasts (x100, CK-14).



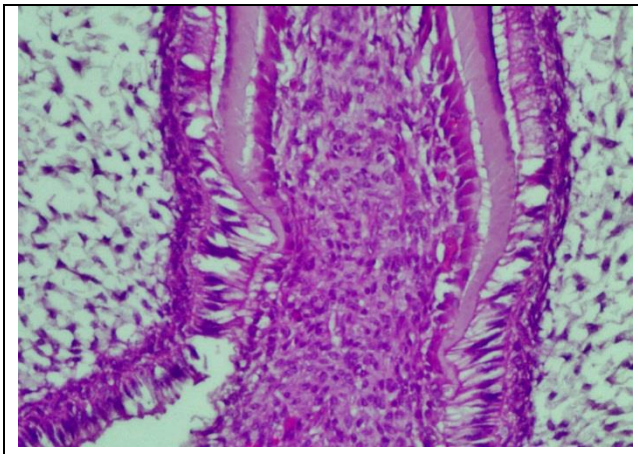
(**Fig-178:** The photomicrograph shows intense staining in preameloblasts (x100, CK-19).



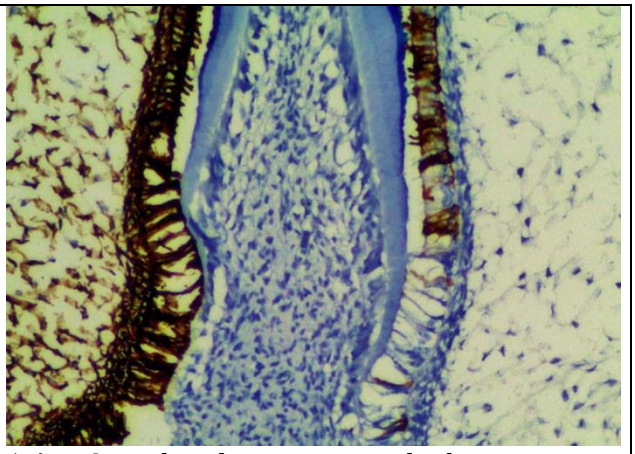
(**Fig-179:** The photomicrograph shows intense staining in preameloblasts (x100, E-Cadherin).

PRESECRETORY AMELOBLASTS

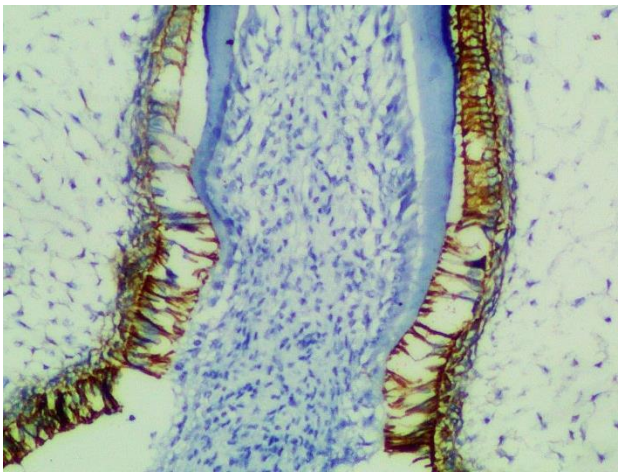
The zone of presecretory ameloblasts is a short segment of cells that extends from the transition point of preameloblasts to transition point of secretory ameloblasts. These cells are more tall columnar shaped cells containing long and slender nucleus with increased cytoplasmic proportion. The nucleus occupies basal 1/3rd of the cell with reversed nuclear polarity but nuclear palisading is not a constant feature (Fig). The cells are bordered by differentiated ectomesenchymal cells (Preodontoblasts) producing dentin matrix on one side and prominent stratum intermedium on the other side. The basement membrane appears disintegrated.(**Fig-180**)



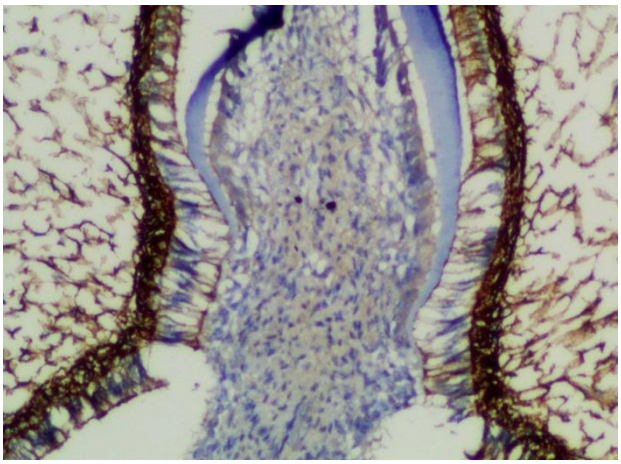
(**Fig-180:** The photomicrograph shows presecretory ameloblasts (x100, H&E).



(**Fig-181:** The photomicrograph shows intense staining in presecretory ameloblasts (x100, CK-14).



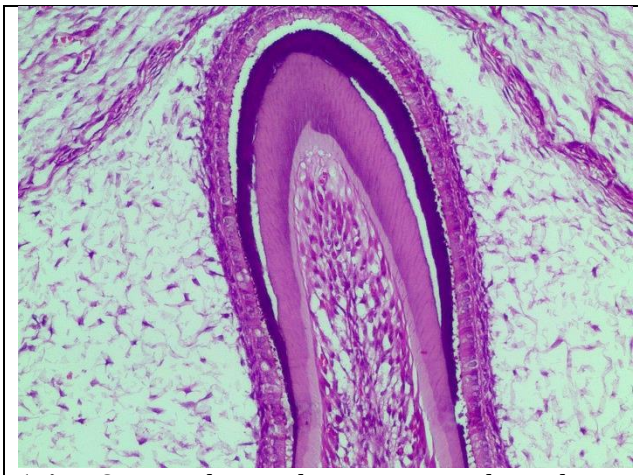
(**Fig-182:** The photomicrograph shows intense staining in presecretory ameloblasts (x100, CK-19).



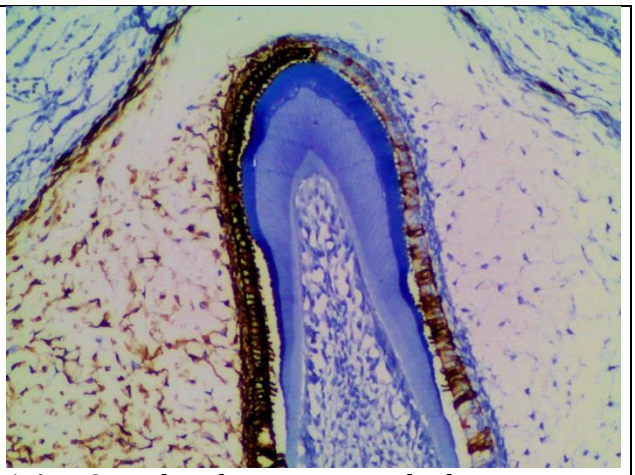
(**Fig-183:** The photomicrograph shows staining in membranous areas of presecretory ameloblasts (x100, E-Cadherin).

SECRETORY AMELOBLASTS

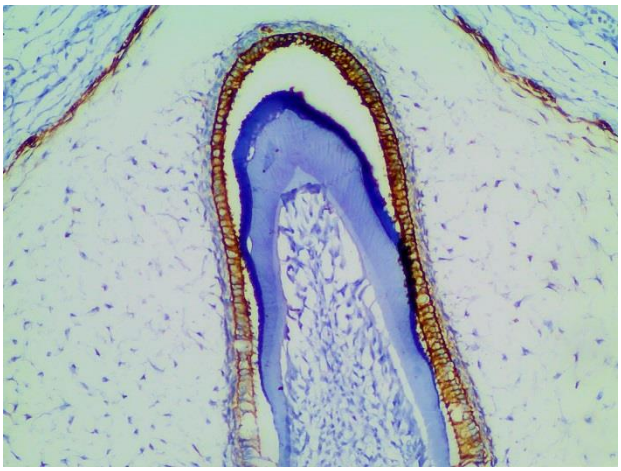
The zone of secretory ameloblast cells extends from the transition point of presecretory ameloblasts on either side. These cells are columnar shaped cells containing round nucleus and conical cytoplasmic projections (Tomes' process). The nucleus occupies basal 1/3rd of the cell with reversed nuclear polarity and palisading. (However, palisading is not a typical feature of initial secretory ameloblasts with Tomes' process). (**Fig-184**) The cytoplasm of the cells appear granular. The cells are bordered by enamel matrix on one side and a thin layer of stratum intermedium on the other side.



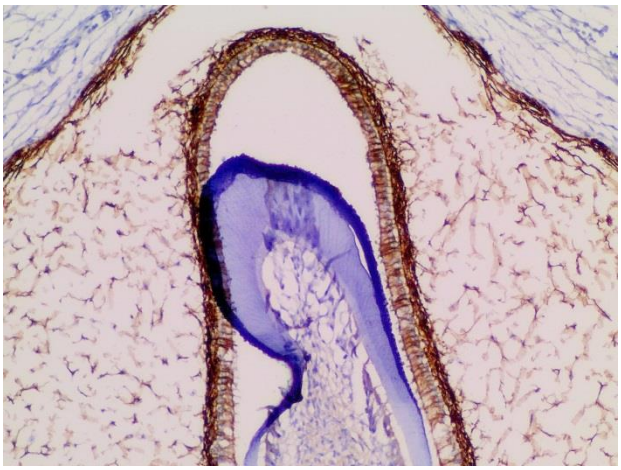
(**Fig-184:** The photomicrograph shows secretory ameloblasts (x100, H&E).



(**Fig-185:** The photomicrograph shows intense staining in secretory ameloblasts (x100, CK-14).



(**Fig-186:** The photomicrograph shows intense staining in secretory ameloblasts (x100, CK-19).



(**Fig-187:** The photomicrograph shows intense staining in secretory ameloblasts (x100, E-Cadherin).

SUMMARY OF THE RESULTS

Ameloblastoma:

The immunoexpression pattern varied among different ameloblastomas in respect to the level of differentiation. The expression pattern is described in the table.

GroupII	**CK-14 expression**	**CK-19 expression**	**E-Cadherin expression**
Group-IIa: regions with inner enamel epithelium like morphology. (Among the 26 examined regions, 10 regions (38.5%) showed outer cells resembling IEE).	All the regions in Group-IIa showed intense CK-14 expression. 10/10 regions were positive.	In Group-IIa, 7 regions did not express CK-19, 2 regions showed mild expression and 1 region showed intense expression. 7/10 regions were negative.	In Group-IIa, 8 regions showed cytoplasmic E-Cadherin expression. 8/10 regions were positive.
Group-IIb: regions with preameloblast like morphology as in early bell stage. (Among the 26 examined areas, 2 regions (7.5%) showed outer cells resembling preameloblasts as in early bell stage).	All the regions in Group-IIb showed intense CK-14 expression. 2/2 areas were positive.	In Group-IIb, 1 region showed intense expression of CK-19. 1/2 areas were positive.	All the regions in Group-IIb showed cytoplasmic expression of E-Cadherin. 2/2 areas were positive.
Group-IIc: regions with preameloblast like morphology as in late bell stage (Among the 26 examined regions, 6 regions (23.1%) showed outer cells resembling preameloblasts as in late bell stage).	All the regions in Group-IIc showed intense expression for CK-14 except one region which had vacuolated cytoplasm. 5/6 areas were positive.	All the regions in Group-IIc were negative for CK-19 expression except, one that showed mild staining. 5/6 areas were negative.	In Group-IIc, only 2 regions showed cytoplasmic expression of E-Cadherin. 2/6 areas were positive.
Group-IId: regions resembling presecretory ameloblast like	All the regions in Group-IId showed intense expression for CK-14.	All the regions in Group-IId showed CK-19 expression with varying	In Group-IId, 3 regions showed immunoexpression for E-cadherin with 2

morphology (Among the 26 examined regions, 4 regions (15.3%) showed outer cells resembling presecretory ameloblasts).	4/4 regions were positive.	intensities. 4/4 regions were positive.	membranous and 1 cytoplasmic staining. 3/4 areas were positive.
Group IIe: regions resembling secretory ameloblast like morphology (Among the 26 regions examined, 4 regions (15.3%) showed outer cells resembling secretory ameloblasts).	All the regions in Group-IIe showed intense expression for CK-14 except one which had vacuolated cytoplasm. 4/4 regions were positive.	In group-IIe, 2 regions showed immunoexpression for CK-19. The cells with vacuolated cytoplasm were negative for CK19 expression. 2/4 regions were positive.	In Group-IIe, 2 regions showed E-Cadherin expression predominantly in the membranous areas. 2/4 regions were positive.

Tooth Germ:

The expression patterns of CK-14, CK-19 and E-Cadherin in the tooth germs were as follows

Group-I	**CK-14 expression**	**CK-19 expression**	**E-Cadherin expression**
Group-Ia: Inner enamel epithelium of early bell stage and late bell stages (8 tooth germs)	All the tooth germs showed intense expression for CK-14. 8/8 tooth germs were positive.	In all the tooth germs CK-19 expression was negative. 8/8 tooth germs were negative.	Among 8 tooth germs, 4 showed cytoplasmic expression of E-Cadherin. 4/8 tooth germs were positive.
Group-Ib Preameloblasts as in early bell stage (2 tooth germs)	All the tooth germs showed intense expression for CK-14. 2/2 tooth germs were positive.	In both the tooth germs, CK-19 expression was minimal. 2/2 tooth germs were positive.	In both the tooth germs, E-Cadherin expression was minimal. 2/2 tooth germs were positive.
Group-Ic: Preameloblasts as in late bell stage (6 tooth germs)	All the tooth germs showed intense expression for CK-14. 6/6 tooth germs were positive.	Among the 6 tooth germs, 5 showed immunoexpression for CK-19. Within the 5 stained tooth germs 3 showed	Among the 6 tooth germs, 4 showed cytoplasmic expression for E-Cadherin. Within the 4 stained tooth germs

		intense staining and 2 showed mild staining. 5/6 tooth germs were positive.	only 1 stained intensively and rest 3 stained slightly. 4/6 areas were positive.
Group-Id: Presecretory ameloblasts (6 tooth germ)	All the tooth germs showed intense expression for CK-14. 6/6 tooth germs were positive.	Among the 6 tooth germs, 4 showed immunoexpression for CK-19. Within the 4 stained tooth germs, 2 showed intense staining and 2 showed mild staining. 4/6 tooth germs were positive.	Among the 6 tooth germs, 2 showed cytoplasmic immunoexpression for E-Cadherin. 2/6 tooth germs were positive.
Group-Ie: Secretory ameloblasts (6 tooth germ)	All the tooth germs showed intense expression for CK-14. 6/6 tooth germs were positive.	All the tooth germs showed intense expression for CK-19. 6/6 tooth germs were positive.	Among the 6 tooth germs, 2 showed cytoplasmic immunoexpression stained for E-Cadherin. 2/6 tooth germs were positive.

DISCUSSION

The morphological assessment was carried out in routine H&E section both in tooth germ and ameloblastoma. This study made it clear the different developmental stages of ameloblasts after analyzing 12 tooth germs (8 tooth germs of late bell stages and 4 tooth germs of early bell stages). The morphological differences between various ameloblasts were described and illustrated in **Chart-I**, as there was no proper study material available that can clearly differentiate various ameloblasts histologically. This study also analyzed the morphology of the outer cells of ameloblastoma islands, compared with the different ameloblasts of tooth germ and categorized them based on the cell structure. This study used immunohistochemical markers to know the expression pattern of ameloblasts of tooth germ and outer cells of ameloblastoma.

The pattern of CK-14, CK-19 and E-Cadherin expression in tooth germ and ameloblastoma are complex. Our study is restricted only to find the expression pattern of the CK-14, CK-19 and E-Cadherin in different developmental stages of ameloblasts of tooth germ and the outer cells of ameloblatomas in order to ascertain morphological and functional differentiation. The immunoexpression pattern revealed that each subtype of ameloblastoma is a homogenous group that comprises tumor cells with heterogeneous differentiation. To the best of our knowledge, no study had categorized ameloblastomas on the basis of outer cell morphology and compared its morphology and immunoexpression with various stages ameloblasts of tooth germ.

CK-14 and CK-19 in tooth germ and ameloblastoma

This study revealed that both CK-14 and CK-19 were expressed in tooth germ and ameloblastoma (Kasper M[49] et al. 1989). Our results showed CK-14 is the main intermediate

filament of ameloblasts of tooth germ and outer cells of ameloblastoma, as all the samples studied showed intense immunostaining regardless of the level of differentiation, except the tumoral cells with vacuolated cytoplasm which did not stain for CK-14 (**Fig-76**). The results in our study varied from the study by Crivelini MM[40] et al. 2003**,** who stated that all the cells of enamel organ of tooth germ expressed CK-14 except secretory ameloblasts (SA) but in our study we could find intense staining of CK-14 in secretory ameloblasts (SA) in all the tooth germs (**Fig-185**). We could relate the difference in CK-14 expression in tooth germ may be due to variation in the gestational age of the fetuses. Regarding ameloblastomas, our study showed similar results with the study by Crivelini MM[40] et al. 2003, as most of the tumor cell expressed CK-14 except the cells with vacuolated cytoplasm.

In our study we could find CK-19 was expressed in preameloblasts (PA-EBS, PA-LBS)(87%) and presecretory ameloblasts (67%) of most of the tooth germs and intensively expressed in secretory ameloblasts (100%) of all the tooth germs (**Fig-178**, **Fig-182** and **Fig-186**), which can be correlated to secretory differentiation, was in accordance to the study by Crivelini MM[40] et al. 2003, but they did not mentioned about presecretory ameloblast stage and staining intensities of various ameloblasts. In contradiction to Crevilini MM[40] et al. 2003, our study revealed 9 out of 26 regions of ameloblastoma (34.6%) with higher level of outer cell differentiation [resembling presecretory (PSA) and secretory ameloblasts (SA)] showed intensive expression of CK-19 (**Fig-23**, **Fig-24** and **Fig-54**).

In agreement with the previous study by Domigues MG[7] et al. 2000, the current study on tooth germ showed CK-19 expression in the preameloblasts (PA-EBS, PA-LBS), presecretory ameloblasts (PSA) and secretory ameloblasts (SA) was associated with secretory differentiation, but Domigues MG[7] et al.2000, did not clearly define different stages of ameloblast of tooth

germ. On contrary, current study did not show down-regulation of CK-14 in secretory ameloblasts in the late bell stages as described by them, which may also be related to the difference in the gestational ages.

In accordance to previous study in dog tooth germ (Nel S[5] et al. 2011), the current study showed CK-19 expression was negative in IEE, positive in PA EBS, PA LBS, PS and SA with more intense staining in SA (**Fig-178**, **Fig-182** and **Fig-186**). On the contrary, our study did not match for CK-14 expression pattern as the presecretory ameloblasts show extensive expression of CK-14.

Our study showed intense CK-14 expression in all ameloblastomas regardless with the level of differentiation was similar to the studies by Babu CN[8] et al. 2010 and Pal SK[39] et al. 2013, which may suggest that tumor cells may retain basal cell characteristics with the potential for proliferation. On contrary to both the previous studies, our present study revealed only 34.6% of ameloblastoma with higher level of outer cell differentiation [resembling presecretory (PSA) and secretory ameloblasts (SA)] showed immunopositivity for CK-19.

Pal SK[39] et al. 2013, studied the expression of CK-14 and CK-19 in mouse tooth germ, for which he didn't specify or illustrate the stage of the tooth germ. They found CK-14 was expressed in entire tooth germ but CK-19 expressed only in dental lamina. Our study can't be compatible as they did not specify the stage of the tooth germ.

A study on rat tooth germ (Osman HI[41] et al. 2006) revealed CK-14 was strongly expressed in IEE of early bell stage, weak or not expressed in the IEE of late bell stage, expressed in the progenitor cells of the cervical loop, strongly expressed in the preameloblasts and ameloblasts during beginning of enamel formation and decreased progressively after complete enamel formation. In contrary, our study in human tooth germ did not show significant

differences in CK-14 expression among various stages of ameloblasts both in early and late bell stages.

Studies by Fukumashi K[42] et al. 2002 and Kumamoto H[6] et al. 2001 revealed CK-19 was expressed in all the cases of ameloblastomas but our study has shown only 34.6% of ameloblastoma regions were immunoreactive for CK-19.

E-Cadherin expression in tooth germ and ameloblastoma

E-Cadherins are calcium-dependent adhesion molecules that play a role in odontogenesis and cytodifferentiation. This study revealed E-Cadherin was expressed both in tooth germ and ameloblastomas, with strong membrane staining in the stellate reticulum of tooth germ and inner (polyhedral) cells of ameloblastomas. (Kumamoto H[4] et al. 1999, Florescu A[53] et al. 2012, Gonzalez-Alva P[54] et al. 2010, and Mello[56] et al. 2013). But, our study is restricted only to find the immunoexpression pattern of various developmental stages of ameloblasts of tooth germ and the outer cells of ameloblastomas only to assess histodifferentiation and morphodifferentiation.

The results of E-Cadherin expression in the ameloblastoma revealed, the expression is predominantly cytoplasmic (80% of regions **Fig-44**) in the ameloblastomas with IEE like outer cell morphology and its expression was down-regulated (50% of regions **Fig-55** and **Fig-104**) when outer cells of ameloblastomas showed differentiation to preameloblasts (PA-EBS, PA-LBS), later the expression of E-Cadherin become predominantly membranous (75% of regions **Fig-43**), when tumoral cells showed differentiation resembling presecretory ameloblasts (PSA) and secretory ameloblasts (SA).

In accordance to the previous studies by Alves P[51]2008 et al and Kumamoto H[4] et al, E-Cadherin was strongly expressed in the cell surfaces of stellate reticulum and stratum intermedium, and slightly expressed in the IEE and OEE of tooth germ.

A study on E-Cadherin expression of human tooth germ (Heymann R[50] et al 2002) reveled, the expression of E-Cadherin was expressed in proliferating inner enamel epithelium but its expression decreased and progressively lost in differentiating ameloblasts, but the other study (Chen M[58] et al. 2007) showed contradictory results. In our study, the E-Cadherin expression is variable on various developmental stages of ameloblasts and does not show any significant inference.

A previous study in mouse tooth germ (Palacios J[52] et al 1995) revealed E-Cadherin was expressed in the proliferating IEE present in the cervical loop, surfaces of polarizing preameloblasts and basal and apical poles of polarized secretory ameloblasts. Another study on the E-Cadherin expression on rat tooth germ (Sorkin C[55] et al. 2000) reveled intense expression in presecretory, transitional, and reduced stage ameloblasts but was dramatically lower in the secretory and maturation stage ameloblasts.

There are conflicting results on E-Cadherin expression in different stages of ameloblasts of tooth germ in our study and among other studies, hence it is necessary to do further research for assessment of its expression both in tooth germs and ameloblastomas.

SUMMARY AND CONCLUSION

1. Cytokeratin-14 is the main intermediate filament of ameloblasts of tooth germ and outer cells of ameloblastoma, as all the samples studied showed intense immunostaining regardless of the level of differentiation, except the tumoral cells with vacuolated cytoplasm.

2. Cytokeratin-19 is expressed in preameloblasts (PA-EBS, PA-LBS), presecretory ameloblasts (PSA) and secretory ameloblasts (SA) of tooth germ, which can be correlated to secretory differentiation. Similarly, the outer cells of ameloblastoma expressed CK-19 in regions that show higher level of cell differentiation [resembling presecretory (PSA) and secretory ameloblasts (SA)].

3. E-Cadherin is expressed in both tooth germ and ameloblastomas, with strong membrane staining in the stellate reticulum of tooth germ and inner (polyhedral) cells of ameloblastomas.

In conclusion, our study indicates that although tumor cells of ameloblastoma have acquired morphodifferentiation to cells resembling secretory ameloblasts (SA) and express the markers of secretory ameloblasts of tooth germ, it does not undergo functional differentiation to the point of enamel formation. Further studies are necessary in large scale with markers such as amelogenin, ameloblastin, fibroblast growth factor and bone morphogenic protein to arrive to a good hypothesis.

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